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Proceedings of the Workshop on Therapeutic Plasmapheresis and Cytapheresis

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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National Institutes of Health

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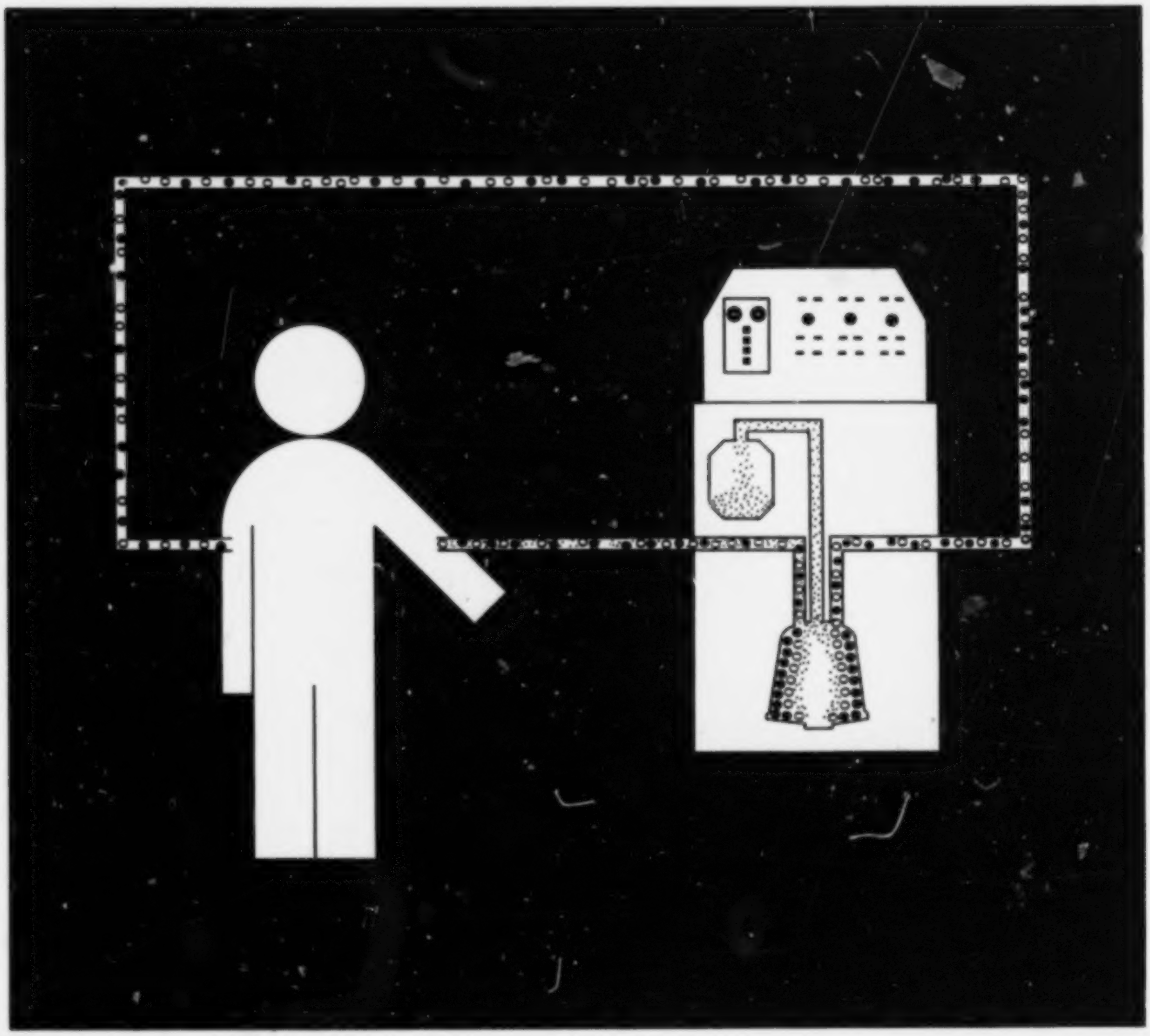

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Proceedings of the Workshop on Therapeutic Plasmapheresis and Cytapheresis

April 25 and 26, 1979

Mann Hall
Medical Sciences Building
Rochester, Minnesota

Sponsored by:
Division of Blood Diseases and Resources
National Heart, Lung, and Blood Institute

Section of Blood Bank and Transfusion Services
Department of Laboratory Medicine
Mayo Clinic and the Mayo Foundation

Editors:
George J. Nemo
and
Howard Taswell

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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PREFACE

A relatively small number of significant technological advances beginning with the discovery of the ABO blood types have permitted the remarkable growth and development of immunohematology and blood banking over the past three-quarters of a century. The discovery and use of anticoagulant and preservative solutions and the use of compatibility testing (particularly the antihuman globulin test) permitted safe transfusion and the subsequent rapid growth of transfusion and blood banking throughout the world. In more recent times, the development of flexible, sterile, disposable, plastic blood containers changed the practice of transfusion therapy from the use of whole blood to the use of specific blood components.

We are now at the beginning of a new era for blood banking which has been made possible by the development and use of centrifugal blood cell separators for plasmapheresis and cytapheresis of donors and patients. Rapidly growing apheresis activities have almost doubled each year over the past five years at our institution as well as at many others. These proceedings focus on the direct treatment of the patient in a variety of diseases that are being treated by plasmapheresis or cytapheresis. A significant portion of the proceedings is devoted to the use of plasmapheresis and plasma perfusion over affinity or absorptive columns that have been selected to remove a specific plasma component, the presence of which is associated with a disease process.

An analogy might be made between the early years of transfusion practice and the early stages of plasmapheresis and cytapheresis which we find ourselves in today. Initially, transfusion practice was limited almost exclusively to treatment with whole blood. In more recent times, with the development of flexible, plastic containers, it has become practical to separate blood into a number of its components which could then be transfused in a component replacement program. With this capability, "shotgun" therapy with whole blood has been fading gradually from transfusion practice. In a few years, we may look back at our present plasmapheresis techniques as "shotgun" therapy also. As with our modern component replacement programs, it would be desirable to develop a component that is associated with a disease process rather than to remove "whole plasma." The many advantages are obvious. Plasmapheresis techniques of this type may soon be possible in certain disease states by combining the use of continuous- or semi-continuous-flow centrifuges with one of several methods of selective removal such as an appropriately chosen affinity column. The last part of these proceedings is devoted to the early and exciting efforts in this direction.

Throughout the proceedings, the authors have attempted to review the current literature pertinent to their subjects and present their own findings. The proceedings also document the subsequent discussions provoked by the questions asked and the problems identified.

Howard Taswell, M.D.
Director, Mayo Clinic Blood Bank
Mayo Clinic
Rochester, Minnesota 55901

INTRODUCTION

The use of therapeutic plasmapheresis and cytapheresis has increased dramatically over the past decade. The increased use of these techniques is due in large part to the development of centrifugal blood cell separators and to a number of reported successes in the treatment of certain disorders with these devices. In 1979 it seemed appropriate to plan a workshop dealing with this rapidly growing research area. The Blood Resources Branch of the Division of Blood Diseases and Resources (National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland) in conjunction with the Mayo Clinic, Rochester, Minnesota, sponsored the Workshop on Therapeutic Plasmapheresis and Cytapheresis which took place at the Mayo Clinic, April 25-26, 1979.

The goals of the workshop were a) to bring together scientists and clinicians to exchange and evaluate recent information related to therapeutic plasmapheresis and cytapheresis and column apheresis using automated continuous and semicontinuous centrifugation systems, b) to identify and analyze problems of mutual concern, and c) to identify fertile areas for individual and collaborative investigation.

The proceedings were prepared from manuscripts submitted by the speakers prior to the workshop. The discussion following each oral presentation was transcribed, edited, and appended to the corresponding manuscript. In the interim between the workshop and publication of the proceedings, the contributors were given an opportunity to update their manuscripts through April 1980 with new, relevant information. For those authors who chose to do so, the information is presented in addenda to their manuscripts. We would like to express our sincere gratitude to the speakers and discussants for making this workshop a complete success. Substantive information was presented and many problems were discussed. It is hoped that as knowledge in the area continues to grow, the role of therapeutic apheresis in the treatment of candidate disorders will ultimately be determined.

George J. Nemo, Ph.D.
Program Manager
Division of Blood Diseases and Resources
National Heart, Lung, and Blood Institute
National Institutes of Health
Bethesda, Maryland 20205

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INTRODUCTORY NOTE

The term "apheresis" derives from the Greek word "aphairesis" (literally, taking off, from aphairen--to take away, from apo + hairein--to take, with the noun ending sis) and refers to the removal of any portion of the vascular fluid, but not usually to the therapeutic removal of whole blood. From this root word and various combining forms have come plasmapheresis, cytapheresis, leukapheresis, lymphapheresis, and plateletapheresis, all of which can be found in indexes and reference catalogues. These spellings conform to the accepted practice in medical terminology of dropping the final vowel of the combining form (cyto-, leuko-, lympho-) when the root word begins with a vowel. Here, the root word begins with "a" and thus "a" is essential to the correct spelling of the word. Dropping the initial "a" from the root word, as is sometimes done inadvertently or incorrectly, can result in confusion with the suffix "-phoresis," as in electrophoresis. Further, it would necessitate indexing and cross-indexing under "p" as well as "a," thus perpetuating a confusing and wasteful practice. The correct spellings have been adhered to throughout the text of this report (except for the charts and graphs). To encourage conformity of usage among researchers, the Division of Blood Diseases and Resources, National Heart, Lung, and Blood Institute, suggests that the correct spelling "apheresis" be used, not only of the root word but in all combined forms.

The editors wish to acknowledge, with grateful appreciation, the literary refinements provided by Mrs. Loretta Allion.

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PROGRAM

Wednesday, April 25, 1979

Morning

(Moderator: Wolf W. Zuelzer, M.D.)

Welcome and Introduction -
Howard F. Taswell, M.D.
Wolf W. Zuelzer, M.D.

"Therapeutic Plasmapheresis and Cytapheresis"
Alvaro A. Pineda, M.D.

"Leukapheresis in Acute Myeloblastic Leukemia"
Janet Cuttner, M.D.

"Lymphapheresis in the Treatment of the Leukemic Phase of Cutaneous T-Cell Lymphoma and Pemphigus Vulgaris"
Richard L. Edelson, M.D.

"Cytapheresis for Procurement of Eosinophils and for the Treatment of Diseases Associated with Eosinophilia"
Gerald J. Gleich, M.D.

Afternoon

(Moderator: Alvaro A. Pineda, M.D.)

"Plateletapheresis in the Treatment of Thrombocythemia"
Edward G. Taft, M.D.

"Plasmapheresis in the Treatment of Hyperviscosity Syndromes and Paraproteinemias"
Jacob Nusbacher, M.D.

"Plasmapheresis in the Treatment of Immune Disease"
Richard F. Branda, M.D.

"Plasma Exchange in Immune Complex Disease and Goodpasture's Syndrome"
Bruce A. Pussell, M.B., F.R.A.C.P.

Thursday, April 26, 1979

Morning

(Moderator: Richard F. Branda, M.D.)

"Plasmapheresis in Neurologic Disorders"
Peter C. Dau, M.D.

"Plasma Exchange for Severe Rhesus (Rh) Disease"
J. F. Graham-Pole, B.M.

"Plasmapheresis in the Treatment of Systemic Lupus Erythematosus"
John Verrier Jones, B.M., F.R.C.P.

"Long-Term Plasma Exchange in Severe Familial Hypercholesterolemia"
Gilbert R. Thompson, B.M., F.R.C.P.

Afternoon

(Moderator: Howard F. Taswell, M.D.)

"Hemoperfusion and Toxin Adsorption in the Treatment of Poisonings"
Michael Gelfand, M.D.

"Charcoal Affinity Column Plasma Perfusion for the Treatment of Intractable Pruritus of Cholestasis"
Bernhard H. Lauterburg, M.D.

"Extracorporeal Immunoadsorbents for Therapy of Autoimmune and Neoplastic Disease"
David S. Terman, M.D.

"Use of Biological Immunoabsorbents in the Treatment of Tumor Host"
S. C. Bansal, M.D.

"Hypercholesterolemia Treatment by Heparin-Agarose Affinity Column"
Bruce A. Kottke, M.D.

Summary

WORKSHOP ATTENDEES

David J. Baird
IBM Corporation
Mt. Kisco, New York

Susan N. Berry
Methodist Hospital
Indianapolis, Indiana

Milan Bier
University of Arizona
Tucson, Arizona

Patricia Blanford
Norton-Children's Hospital
Louisville, Kentucky

Neil Blumberg
Connecticut Red Cross
Blood Services
Farmington, Connecticut

Julian P. Breillatt
E. I. Du Pont de Nemours
and Co., Inc.
Wilmington, Delaware

Leslie Ray Bryant
Southern Baptist Hospital
New Orleans, Louisiana

Roger C. Bucknall
University of Alberta
Edmonton, Alberta, Canada

George W. Buffaloe
Cobe Laboratories, Inc.
Lakewood, Colorado

D. H. Buchholz
Travenol Laboratories, Inc.
Round Lake, Illinois

Richard Cable
Syracuse, New York

Kathleen Cardillo
New England Deaconess
Hospital Blood Bank
Newton Highlands, Massachusetts

Franco Castino
American Red Cross
Bethesda, Maryland

Louise W. Chandler
James E. Holmes
Regional Medical Center
Melbourne, Florida

Amoz I. Chernoff
National Heart, Lung, and
Blood Institute
National Institutes of Health
Bethesda, Maryland

Louise M. Chiaretti
Hospital of the
University of Pennsylvania
Philadelphia, Pennsylvania

Michael Chopek
University of Minnesota Hospital
Minneapolis, Minnesota

William F. Clark
Victoria Hospital
London, Ontario, Canada

Joan C. Craig
Fifth and Broune Medical Center
Spokane, Washington

John T. Crosson
Hennepin County Medical Center
Wayzata, Minnesota

Diane DeRudder
Aurora Area Blood Bank
Aurora, Illinois

Daniel A. Dietrich
St. Lukes Episcopal Hospital
  Blood Bank
Houston, Texas

William A. Dittman
Sacred Heart Medical Center
Spokane, Washington

Michael R. Downing
Los Angeles, California

Raymond B. Duggins
E. I. Du Pont de Nemours
  and Co., Inc.
Wilmington, Delaware

John J. Egan
Wilmington Medical Center
Wilmington, Delaware

Richard B. Eisenberg
Regional Clinical Laboratories
  of N.W. Pennsylvania
Erie, Pennsylvania

Paul Etzell
Fargo Clinic
Fargo, North Dakota

Keelin T. Fry
E. I. Du Pont de Nemours
  and Co., Inc.
Wilmington, Delaware

Ruth Ann Fulton
St. Anthony's Hospital
St. Petersburg, Florida

James W. Funkhouser
Community Blood Center
Dayton, Ohio

David Gibson
Santa Barbara Medical
  Foundation Clinic
Santa Barbara, California

Ronald O. Gilcher
Oklahoma Blood Institute
Oklahoma City, Oklahoma

Dennis Goldfinger
Cedars-Sinai Medical Center
Encino, California

P. A. Gordon
University of Alberta Hospital
Edmonton, Alberta, Canada

Steven A. Gould
Michael Reese Hospital
Chicago, Illinois

Gerald A. Grode
Fenwal Laboratories, Inc.
Round Lake, Illinois

Larry Grossman
Vancouver General Hospital
Vancouver, B.C., Canada

Claes-Ake Gulberg
Gambro, Inc.
Barrington, Illinois

Theresa Haddy
National Heart, Lung, and
  Blood Institute
National Institutes of Health
Bethesda, Maryland

Thomas Hallin
Methodist Hospital
St. Louis Park, Minnesota

Challen E. Hammer
Albany Medical Center Hospital
Albany, New York

James E. Hart
Richmond Metropolitan Blood
  Service
Richmond, Virginia

Robert Hawkins
University of Michigan
Ann Arbor, Michigan

Kenneth Hou
AMF Inc.
Meriden, Connecticut

Judith Houx
Baxter Travenol Laboratories
Round Lake, Illinois

William J. Hunter
University of Kansas
Medical Center
Kansas City, Kansas

David P. Huston
National Institute of Arthritis,
Diabetes, and Digestive and
Kidney Diseases
National Institutes of Health
Bethesda, Maryland

Barbie Jenks
St. Lukes Episcopal Hospital
Blood Bank
Houston, Texas

David C. Jenkins
Community Blood Center of
Greater Kansas City
Kansas City, Missouri

Frank R. Jones
University of Washington
Seattle, Washington

Melanie S. Kennedy
Ohio State University Hospitals
Columbus, Ohio

Amanullah Khan
Wadley Institutes of Molecular
Medicine
Dallas, Texas

Gordon F. Kingsley
Haemonetics Corporation
Braintree, Massachusetts

John A. Koepke
University of Iowa
Iowa City, Iowa

Todd A. Kolb
South Carolina Red Cross
Blood Services
Gadsden, South Carolina

Peter Konstantin
Gambro, Inc.
Barrington, Illinois

Sheldon Krasnow
Oak Park, Illinois

Joan Kumar
Massachusetts General Hospital
Boston, Massachusetts

F. Lynn Leverett
South Miami Hospital
Miami, Florida

Joshua Levy
U.C.L.A. Medical Center
Los Angeles, California

Richard T. Light
University of Minnesota
Minneapolis, Minnesota

Abraham T. T. Lin
St. Paul Regional Red Cross
Blood Center
St. Paul, Minnesota

Marsha Linden
Hennepin County Medical Center
Shoreview, Minnesota

Don D. Lobdell
Cobe Laboratories, Inc.
Lakewood, Colorado

Bruce C. McLeod
Rush-Presbyterian-St. Lukes
Medical Center
Chicago, Illinois

B. Daniel McLeod
Calgary General Hospital
Calgary, Alberta, Canada

Robert P. Mappes
IBM Corporation
Mt. Kisco, New York

Maximo Margulies
Centro de Investigaciones
Buenos Aires, Argentina

Tom Mark
Mt. Sinai Medical Center
Miami Beach, Florida

Atilia M. Martinez
University of California,
Irvine Medical Center
Costa Mesa, California

Joseph J. Mazza
Marshfield Clinic
Marshfield, Wisconsin

Jay E. Menitove
Milwaukee Blood Center, Inc.
Milwaukee, Wisconsin

Richard Meyst
Fenwal Laboratories, Inc.
Round Lake, Illinois

Erik K. Mitchell
S.U.N.Y. Upstate Medical Center
Syracuse, New York

Richard D. Murdock
Haemonetics Corporation
Braintree, Massachusetts

George J. Nemo
National Heart, Lung, and
Blood Institute
National Institutes of Health
Bethesda, Maryland

Uif Nylen
University of Lund
Lund, Sweden

Mary Kay Ochal
North Suburban Blood Center
Glenview, Illinois

Thomas O'Neill
Mt. Auburn Hospital Blood Bank
Cambridge, Massachusetts

Adelisa Panlilio
American Red Cross
Nashville, Tennessee

C. Allen Payne
Michigan Community Blood Center
Grand Rapids, Michigan

Marian J. Peterson
Aurora Area Blood Bank
Aurora, Illinois

Herbert F. Polesky
Minneapolis War Memorial
Blood Bank
Minneapolis, Minnesota

P. K. Ray
Medical College of Pennsylvania
Philadelphia, Pennsylvania

Leo E. Reilly
St. Anthony's Hospital
St. Petersburg, Florida

Anthony A. Rene
National Heart, Lung, and
Blood Institute
National Institutes of Health
Bethesda, Maryland

Curt A. Ries
University of California
San Francisco, California

Eloys Rieser
St. Anthony Medical Center
Crown Point, Indiana

Mario Ritter
Johnstown Regional Red Cross
Blood Services
Johnstown, Pennsylvania

David Root
Millipore Corporation
Bedford, Massachusetts

David Rousculp
IBM Corporation
Princeton, New Jersey

Patricia Sechrest
U.C.I. Medical Center
Orange, California

Paul J. Schmidt
Southwest Florida Blood Bank, Inc.
Tampa, Florida

Ralph A. Schmitz
Community Blood Center of Greater Kansas City
Kansas City, Missouri

Jeffrey Shideman
Gambro, Inc.
Barrington, Illinois

Sherrill Slichter
Puget Sound Blood Center
Seattle, Washington

James Smith
Cleveland Clinic
Cleveland, Ohio

Judith A. Smith
University of Minnesota Blood Bank
Minneapolis, Minnesota

Sam Sofer
University of Oklahoma
Norman, Oklahoma

Cathy Spadaccini
Hennepin County Medical Center
Minneapolis, Minnesota

Wayne S. Stebbins
St. Anthony's Hospital
St. Petersburg, Florida

Patti Stevens
Victoria Hospital
London, Ontario, Canada

Michael R. Sullivan
Fenwal Laboratories, Inc.
Deerfield, Illinois

Harry L. Taylor
North Jersey Blood Center
East Orange, New Jersey

Helen Tochen
Pacific Northwest Region Blood Services
Portland, Oregon

Geraldine M. Turner
James E. Holmes Regional Medical Center
Palm Bay, Florida

Joel Umlas
Mount Auburn Hospital
Cambridge, Massachusetts

Thiru Vaithianathan
American Red Cross Blood Services
Chicago, Illinois

Bernie Van Wie
University of Oklahoma
Norman, Oklahoma

Winston Wo
U.C.L.A. Medical Center
Los Angeles, California

Alan Wong
Hunter Blood Center
Clearwater, Florida

Daniel Wright
National Cancer Institute
National Institutes of Health
Bethesda, Maryland

Alma Young
University of Texas Medical Branch at Galveston
Galveston, Texas

Jeffry Young
University of Washington
Seattle, Washington

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THERAPEUTIC PLASMAPHERESIS AND CYTAPHERESIS

Alvaro A. Pineda, M.D.
Howard F. Taswell, M.D.
S. B. Moore, M.B., B.Ch.
Mayo Clinic
Rochester, Minnesota

INTRODUCTION

The use of plasmapheresis and cytapheresis as techniques to treat or modify disease processes has increased dramatically since the advent of continuous and semicontinuous flow centrifugation technology. This technology permits separation of the blood components on the basis of their differences in density in a gravitational field created in a rotating centrifuge bowl. It has been applied, with varying degrees of success, to the treatment of conditions characterized by an excess of specific blood components that can readily be removed from the bloodstream. The removal of specific blood components in combination with replacement therapy results in a new overall concept, that of reconstructive manipulation of an abnormal blood supply to reestablish normalcy.

This paper describes therapeutic plasmapheresis and cytapheresis applied in a variety of situations in which such procedures were of variable clinical value.

PLASMAPHERESIS

Intensive plasmapheresis amounting to at least a total plasma exchange was employed for the treatment of malignant paraproteinemias and cryoglobulinemias, cold agglutinin autoimmune hemolytic anemia, coagulation factor inhibitors, Goodpasture's syndrome, and thrombotic thrombocytopenic (TTP), idiopathic thrombocytopenic (ITP), and post-transfusion purpuras. In the last 2 years, 185 total plasma exchanges (TPE's) were performed in a total of 47 patients. The average volume removed was 4,915 ml. The replacement fluid of choice was a 5 percent solution of normal serum albumin used in a majority of procedures. Purified protein fraction and fresh frozen plasma were used occasionally; the latter in cases where replacing clotting factors was indicated. Generally, 75 percent of the removed volume was replaced with protein solution and 25 percent with crystalloids, preferentially 0.9 percent solution of NaCl. Sodium citrate was the anticoagulant used in all procedures. The majority of TPE's used a semicontinuous flow centrifugation system (Haemonetics); the continuous flow system (NCI-IBM) was used preferentially in pediatric or small adult patients (less than 100 pounds body weight). In 15 procedures (8% of the total) there was a reaction. There were 13 cases of mild to moderate hypotension associated with lightheadedness; the reaction responded promptly to a rapid infusion of replacement solutions. Two reactions were characterized by urticaria and wheezing; one reaction was severe enough to require administration of epinephrine intravenously because of severe bronchospasm and hypotension. The patient was later found to have an anti-IgA antibody of limited specificity and did not react when infused with IgA deficient plasma or plasma derivatives. The average duration of the procedure was 3.32 hours. A water-bath in-line blood warmer (Hemokinetitherm) was used routinely to warm blood during infusion. In addition, the apheresis room temperature was raised to 80 °F when cryoglobulinemia patients were exchanged.

TPE in Malignant Paraproteinemia and Hyperviscosity Syndrome

TPE is currently considered the treatment of choice for removal of paraproteins and hyperviscosity syndrome. Data obtained from the treatment of 15 patients with paraproteinemia and hyperviscosity syndrome are summarized in figures 1, 2, and 3. TPE resulted in a reduction of total proteins of 28 $\pm$7 percent and 52 $\pm$15 percent reduction of serum viscosity as seen in figures 1 and 2. As expected, efficiency of removal was greater for IgM (63%) than for IgA (50%) and IgG (43%) (figure 3). In all patients, TPE rapidly arrested bleeding and controlled other manifestations associated with hyperviscosity. The exchange was particularly useful in the immediate preoperative preparation of two patients who needed emergency surgery for coincidental pathologic processes. The beneficial aspects of this therapy have been emphasized in previous publications (1,2,3).

TPE in Cryoglobulinemia

Four patients with monoclonal cryoglobulinemia (three IgG and one IgM) have been treated with TPE in conjunction with chemotherapy. Three patients with IgG lambda cryoglobulin, Raynaud's phenomenon, arthralgias and extensive blistering and ulceration of the skin responded very well to the combined TPE and chemotherapy modality. TPE was followed by marked symptomatic relief of arthralgias and Raynaud's phenomenon, reduction of the

Figure 1.--Total plasma exchange: Paraproteinemia and hyperviscosity

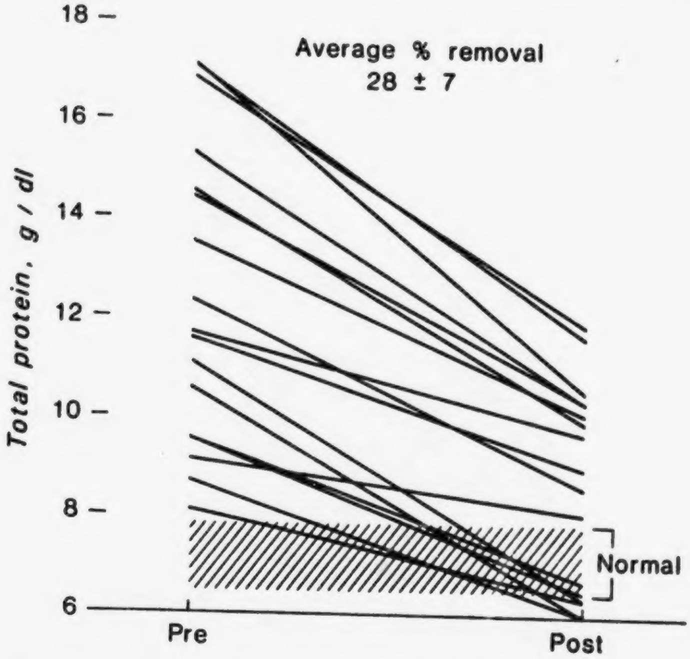
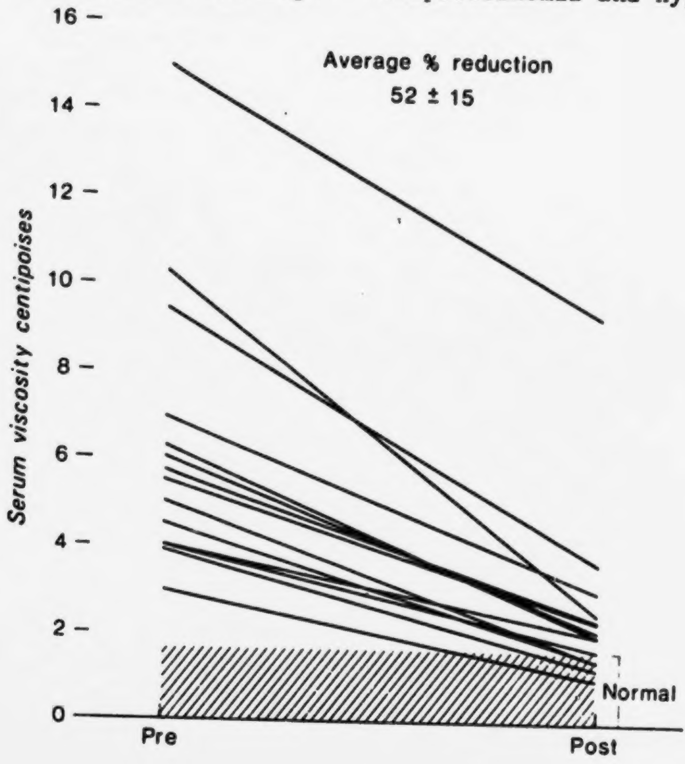
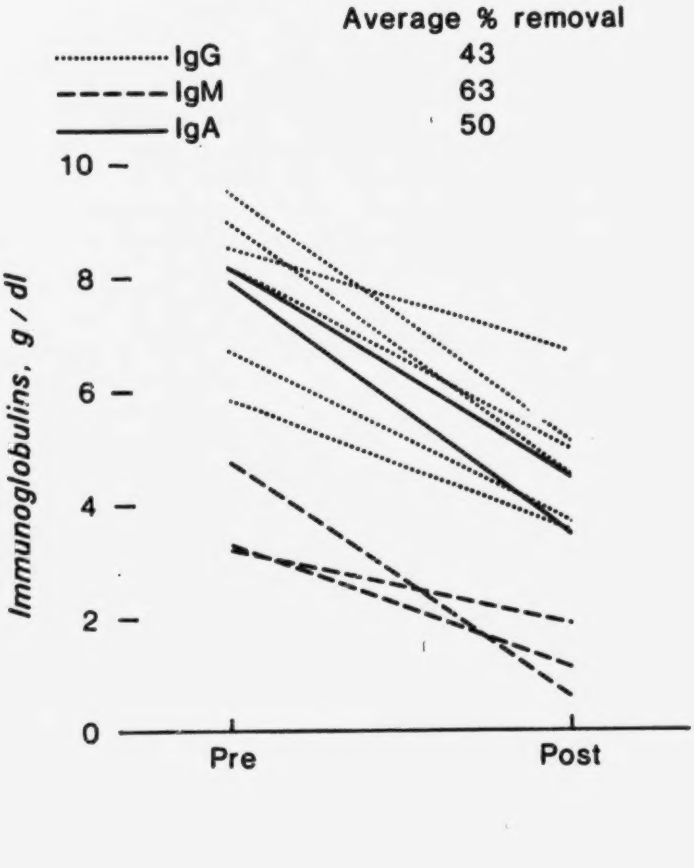


Figure 2.--Total plasma exchange: Paraproteinemia and hyperviscosity



extent of skin necrosis, and no further blistering. Two patients have required long-term therapy, needing an exchange every 3 or 4 weeks in one case and conventional bag plasmapheresis (2 liter) every 2 weeks in the other; in both cases, discontinuation of plasmapheresis has resulted in skin ulcerations despite continuous chemotherapy. Initial TPE's resulted in a reduction of IgG of 56 to 66 percent of original levels and cryocrits were reduced 6 to 7 percent per procedure. In one patient, extensive necrosis of soft tissues in the lower extremities necessitated an above-knee amputation and multiple debridements. This patient had a cryoglobulin with a very high thermal range, liquefying at 34 °C and solidifying at 1 °C. The serum viscosity went from 2 centipoises at 26 °C to 7 at 0 °C. Four TPE's were done in 2 months, reducing a cryocrit of 11 percent to trace by the time of discharge. In the patient with IgM cryoglobulinemia, hyperviscosity symptomatology was predominant; a reduction of 67 percent of original cryoglobulin was achieved with one exchange, resulting in marked amelioration of symptoms of hyperviscosity, bleeding, and arthralgias.

Figure 3.--Total plasma exchange: Paraproteinemia and hyperviscosity

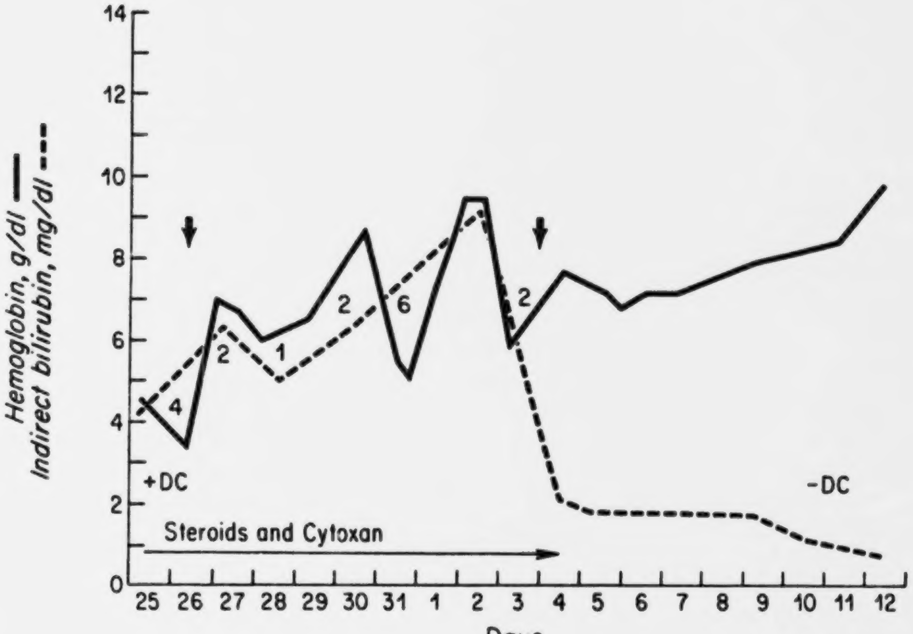


Cryoproteins are thought to cause impaired peripheral circulation and cold urticaria. Russell and coworkers have reported the healing of ischemic ulcer associated with cryoglobulinemia after plasma exchange (1). Our experience supports such a finding and we feel that TPE and chemotherapy limit the extent of necrosis and facilitate healing of lesions. It has been suggested that it may not be necessary to remove the cryoglobulins completely since partial reduction in their concentration lowers the temperature required to induce their precipitation.

TPE in Cold Agglutinin Autoimmune Hemolytic Anemia

Two patients with severe hemolytic process, positive direct antiglobulin test, and circulating cold agglutinins with anti-I specificity were treated with TPE. Figure 4 depicts the course in a 21-year-old female who, following an upper respiratory infection, developed severe hemolysis requiring up to four units of blood in 24 hours to maintain hemoglobin level at 6 g/100 ml. Below this level she showed signs of cardiac decompensation.

Figure 4.--Total plasma exchange: Cold agglutinin induced hemolysis



Each arrow indicates a TPE, the number of units of red cell are indicated on the graph. DC = Direct Coombs

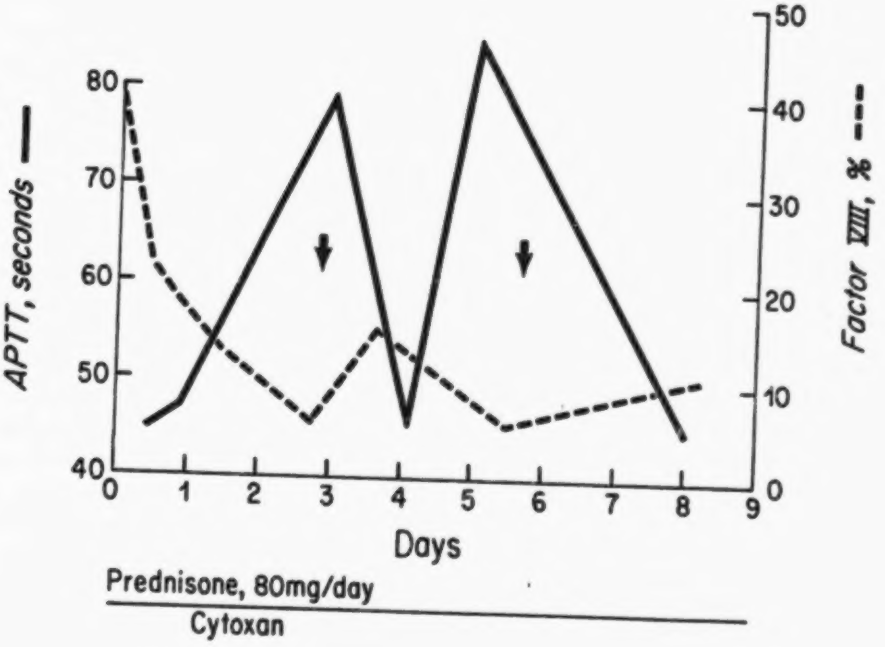
She had been on steroids for 2 weeks prior to first exchange; following the first exchange, her blood requirements diminished until relapse, which prompted a second exchange followed by recovery. The patient had a low titer (1:64) high thermal amplitude antibody. The second patient likewise showed significant lessening of hemolysis with decreased blood requirements and a reduction in antibody titer from 1:4,096 to 1:1,024. This patient, a 76-year-old man, had a 4-year history of well-compensated cold agglutinin disease. Two weeks prior to admission, the patient had developed mycoplasma pneumonia followed by brisk hemolysis; hemoglobin could not be maintained above 6 g/dl despite transfusion of 3 units of red blood cells daily prior to the exchange. Following it, the patient reverted back to cold agglutinin disease with a mild hemolytic process requiring no blood transfusions.

A case of a patient with Evans syndrome, in whom hemolytic anemia was associated with a warm autoantibody as well as a low titer high thermal amplitude cold agglutinin, has been reported (23). The authors concluded that plasmapheresis had been a safe and effective therapy in the management of immune thrombocytopenia and autoimmune hemolytic anemia. They observed a significant decrease of blood transfusion requirements and the patient seemingly went into remission for 37 days following three 3,000 ml exchanges. A case has also been reported (15) in which plasma exchange stabilized hemoglobin levels and decreased Coombs reactivity following plasma exchange, in a patient with immune hemolytic anemia.

TPE for the Treatment of Idiopathic Coagulation Factor Inhibitors

Four such patients, one with Factor V and three with a Factor VIII inhibitor, were treated with immunosuppression and total plasma exchange, ranging from one exchange for the Factor V to two, three, and five exchanges for the Factor VIII inhibitors. Treatment resulted in cessation of bleeding, lowering of inhibitor activity and normalization of coagulation tests in three patients. A fourth patient showed a marked reduction of APTT (162 seconds to 84). One patient with Factor VIII inhibitor was reported in detail (4). A second patient responded very well to two exchanges with no progression of subcutaneous bleeding or development of bleeding in new sites. The patient was dismissed 5 days after the second exchange and continued to be treated with steroids and Cytoxan. He died 7 days following dismissal; the cause of death is unknown. The patient's course, reflected by coagulation test data, is illustrated in figure 5. A third patient with Factor VIII inhibitor was admitted with hematomas of lower and upper extremities associated with bleeding into the neck and a sublingual hematoma with oozing into the mouth. The latter was brought under control by exchange and, with the exception of a large hematoma at the venipuncture site occurring during last exchange, no further bleeding was observed. The Factor VIII inhibitor activity was reduced from 125 U to 23 U (Bethesda) following five exchanges. In these exchanges, no Factor VIII concentrate or fresh frozen plasma was used as replacement, a departure from the technique employed in the previous cases. Our experience closely parallels that cited by other groups (5,6).

Figure 5.--Total plasma exchange



Each arrow indicates a TPE.

TPE in Goodpasture's Syndrome

Six patients with Goodpasture's syndrome proven by histology were treated with TPE in conjunction with immunosuppressive therapy. The five patients who presented with severe hemoptysis and respiratory manifestations showed dramatic improvement with this therapy. Antiglomerular basement membrane antibody titers were lowered in all six patients and renal function improved in the three nonanuric patients. TPE and immunosuppression did not reverse severe renal failure in the three anuric patients. Our experience with TPE and immunosuppression for the treatment of Goodpasture's syndrome presenting with severe pulmonary involvement is in marked contrast to previous experience prior to the introduction of TPE. Data are summarized in table 1.

Table 1.--Clinical features and outcome

			Initial			
Patient	Sex	Age (yrs.)	Creatinine (mg/dl)	Hbg. (gm/dl)	Plasmapheresis	Outcome
1	F	54	12.8	5.8	—	Chronic hemodialysis: unsuccessful transplant.
2	M	22	12.7	5.0	11/28 days	Successful transplantation.
3	F	53	9.1	9.0	8/17 days	Died after 12 months on chronic hemodialysis.
4	M	24	1.3 (Peak 1.5)	7.1	11/29 days	Full recovery.
5	M	27	1.0 (Peak 1.1)	8.6	18/26 days	Full recovery.
6	F	19	2.2	10.3	7/12 days	Full recovery.

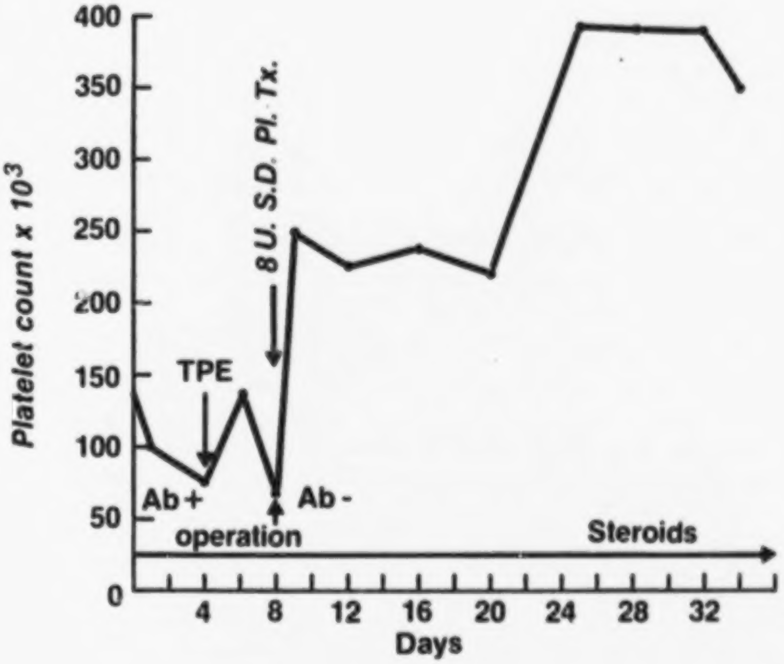
All patients received prednisone 60 mg/d + cyclophosphamide 2 mg/kg except patient 6 who received only prednisone and patient 1 who received neither.

Encouraging results in preserving both pulmonary and renal function have recently been reported by Lockwood's group (7,8,9) and reproduced by others (10,11,12). The rationale of this therapy is to remove anti-GBM antibodies by exchange while at the same time preventing their production by immunosuppression. Our series emphasizes the potential for recovery using exchange and immunosuppression and demonstrating the usefulness of TPE in controlling the pulmonary aspect of the syndrome. Our patients fulfilled the following diagnostic criteria: bilateral pulmonary infiltrates and/or hemoptysis, anemia, anti-GBM antibody, and glomerulonephritis; so did one other patient who recovered fully without immunosuppression or plasmapheresis (13).

TPE for the Treatment of TTP, ITP, and Post-Transfusion Purpura

Our experience is limited to the treatment of three patients, one with each disorder. In the case of ITP, the exchange was performed preoperatively in a 53-year-old woman with ITP diagnosed 8 years prior to admission. The patient underwent splenectomy, having responded poorly to steroid therapy, and at that time she bled copiously as she did while undergoing tooth extractions in the past, requiring transfusion of blood and platelets. She had an acoustic neurinoma which necessitated craniotomy and resection. Figure 6 illustrates the patient's course with preexchange platelet count and response to exchange. The patient did not experience bleeding intra- or postoperatively but received prophylactically two platelet transfusions of four units each from a donor chosen on the basis of failure of patient's serum to lyse donor platelets labeled with ^{51}Cr in an in vitro assay. Such assay, a modification of a previously reported test (14), demonstrated a platelet antibody before pheresis which could not be detected following exchange. As can be seen on figure 6, a platelet increment was observed following exchange, exceeding the increment anticipated by the transfusion of eight units of platelets. Such effect was sustained for at least 24 days after exchange.

Figure 6.--TPE in ITP



Each arrow indicates a lymphapheresis.

Two cases of immune thrombocytopenia have been reported previously (15). In one, as in our case, TPE was followed by an increased platelet count and what seemed to be a complete remission. In the other case, no improvement was reported following one exchange. A similar claim of remission has been made by another group (15a). Gratifying results were reported by Marder et al. (16) in three nonsplenectomized patients with ITP, whose counts rose over 100,000 within 48 hours following plasma exchange after discontinuation of steroid therapy. Two other patients did not respond favorably, and an asplenic patient responded initially, relapsed after 31 days, and then responded favorably a second time. All of the four responsive patients have stayed in remission for over 4 months.

The patient with TTP was a 28-year-old woman who developed headaches, easy bruising, and nosebleeds 2 weeks following an upper respiratory infection. She rapidly developed severe thrombocytopenia associated with slurred speech, facial drooper, left upper extremity numbness, and weakness. The diagnosis was confirmed histologically on bone marrow and peripheral lymph node biopsies. She was treated with nine total plasma exchanges (the last two using fresh frozen plasma replacement), antiplatelet drugs, and steroids. Following the second exchange, there was definite improvement of neurologic manifestations and an increase of platelet count; however, it could not be sustained. Seven additional exchanges were required, as illustrated in figure 7. The patient has had no recurrence of symptomatology and her hemoglobin and platelet count have been normal since discharge and 3 months later. Two additional cases, both confirmed histologically and, like the previous case, lacking the platelet aggregating factor, described by Lian et al. (17) have failed to respond to plasma infusions but have responded rapidly to TPE with a significant elevation of platelet counts over 100,000 and marked clinical improvement. Several forms of therapy have been advocated for TTP. Fresh frozen plasma infusions have been deemed efficacious (17). They did not have effect on clinical status and platelet counts in our patients; failure of FFP infusions has been reported previously (18). Therapeutic successes of exchange transfusion (19,20) and plasmapheresis (6,21) have been emphasized in recent reports. Thrombotic thrombocytopenic purpura may be a spectrum of disease or a syndrome with many diverse causes, which may be altered by diverse therapeutic approaches. Removal of a toxic substance by plasmapheresis has been postulated as a reason for the effectiveness of such treatment (21).

Finally, the course of a patient with post-transfusion purpura is illustrated in figure 8. This patient, a 65-year-old man, presented with a history of poorly defined myeloproliferative disorder with baso- and eosinophilia and anemia. The patient had two well-documented transfusion reactions characterized by precipitous drop of platelet count associated with a diffuse erythematous and purpuric rash of extremities and trunk in the past. Forty-eight hours after the transfusion of two units of frozen-thawed erythrocytes, the patient developed a precipitous drop of platelet count associated again with a purpuric rash and petechiae on extremities and trunk; his platelet count dropped from 140,000 platelets/mm^3 to 33,000 in

72 hours, and 8 days later down to 20,000 with petechiae but no other evidence of bleeding. A total exchange was performed, at which time six units of single donor platelets were administered. Platelet antibody activity decreased by 54 percent when comparing percentage of radioactivity released between samples taken before and after exchange and assayed in parallel with the same target platelets. The platelets of the donor utilized for transfusion, unlike platelets from five other donors, had not been lysed in an in vitro ^{51}Cr release assay. A 60,000 platelet increment/mm^3 was observed after exchange and platelet transfusion. A gradual decline to preexchange level was observed 6 days after exchange; at that time a second platelet transfusion using the same donor resulted in a 30,000 increment/mm^3. No further reactions were observed after the exchange, despite the fact that the patient was transfused on several occasions with red cell products. Plasmapheresis with removal of the antibody proved to be effective therapy in a case (22) where antibody activity as measured by ^{51}Cr release disappeared following plasmapheresis, paralleling clinical improvement and increasing platelet counts to normal levels. Three manual plasmaphereses were performed in this case with a total plasma removal of 4,900 ml.

Figure 7.--TPE in TTP

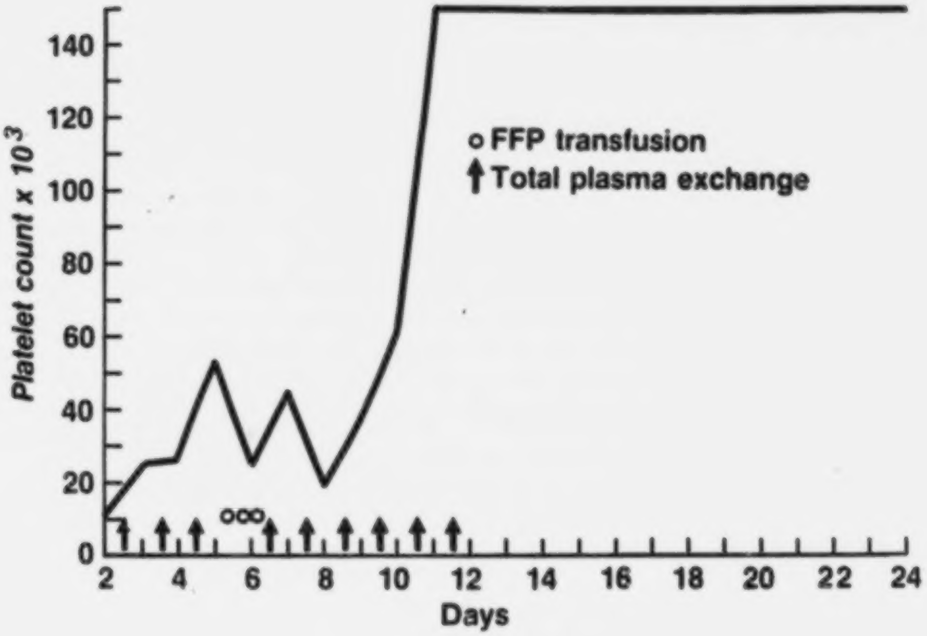


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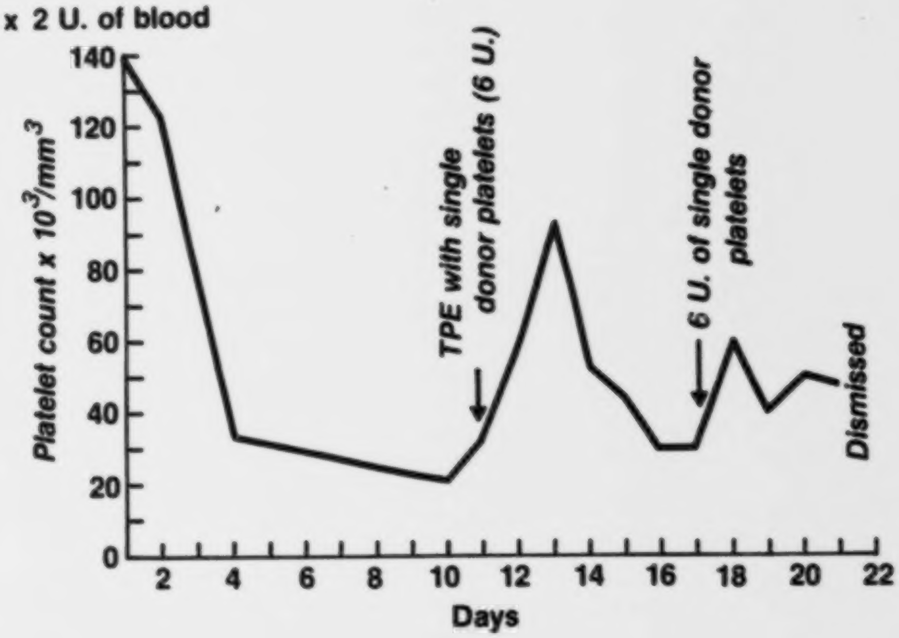
Figure 8.--TPE in post-transfusion purpura



CYTAPHERESIS

The continuous- and semicontinuous-flow centrifugation system separates whole blood into red blood cells, white blood cells, platelets, and plasma. The white blood cells, or leukocyte fraction, can be prepared with a predominance of either lymphocytes or granulocytes with varying amounts of platelet and red cell contamination. The leukocyte fractions will have a predominance of eosinophils or blast cells when these cells happen to circulate in excessive numbers. Cytapheresis is defined as the removal of a large number of leukocytes or platelets from the patient's bloodstream with the return of all remaining components. In theory, the removal of leukocytes might create a gradient between extravascular and intravascular pools, resulting in a net removal of cells from tissue depots.

LYMPHAPHERESIS

Leukapheresis for the removal of lymphocytes or Sézary cells was performed a total of 29 times for the treatment of three patients with lymphosarcoma cell leukemia (LCL) and five patients with Sézary syndrome.

Figures 9 and 10 illustrate the results of 14 lymphaphereses in three patients with LCL. Figure 10 shows the effect of seven removals leading to a count of 46,000, at which time a small dose of steroids was administered. The patient had a short-lived remission. He had refused further chemotherapy, having undergone it off and on for the preceding 3 years. Lymphapheresis was undertaken to minimize the dose of the therapeutic agent and ameliorate its adverse effects. The other two patients had severe thrombocytopenia precluding chemotherapy. Lymphapheresis was undertaken again to reduce tumor load and, consequently, dosage of chemotherapeutic agent. Reduction in counts was associated with subjective clinical improvement, and the patients received a reduced dose of chemotherapeutic agents. One patient, whose course is illustrated in figure 9, underwent two consecutive lymphaphereses on two occasions with a 3-month interval between treatments. None of the patients treated had a significant reduction in platelet counts following cytapheresis. The overall results in this group of patients are as follows: 14 lymphaphereses with an average duration of 3.03 hours (range 2.30 hours to 4.00 hours) resulted in the removal on the average of 8.7 x 10^{11} lymphocytes per procedure (range 26% to 85%). There were no complications associated with the procedure, and, with the exception of two mild hypotensive episodes in one patient, the procedures were well tolerated.

Figure 9.--Lymphapheresis in LCL

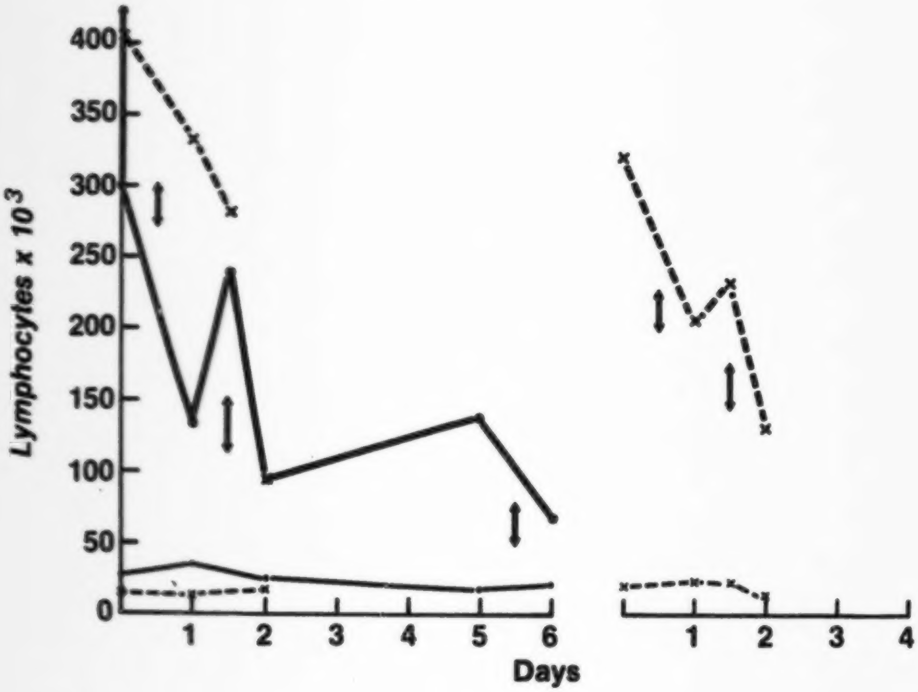
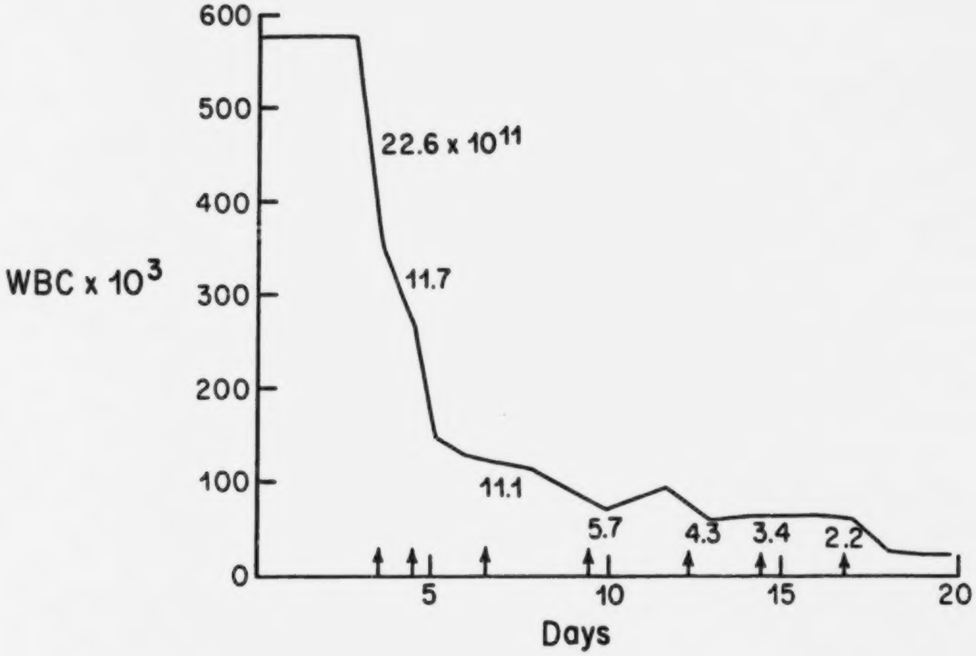


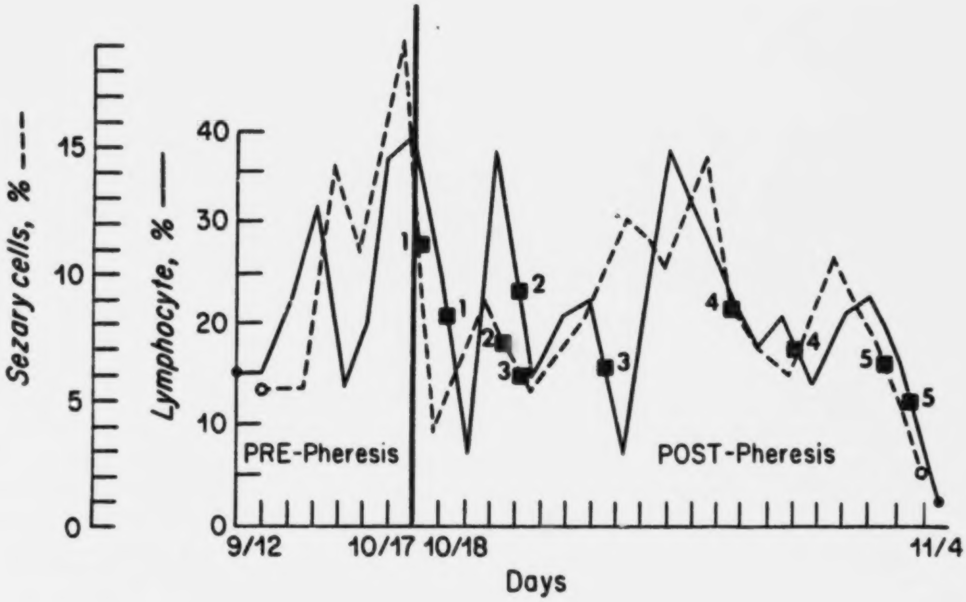
Figure 10.--Lymphapheresis



Objective regression of chronic lymphocytic leukemia induced by intensive lymphapheresis has been reported previously (24), suggesting it as a potentially useful treatment for CLL. After leukapheresis therapy in that study, the median doubling time of the lymphocyte count was 71 days, demonstrating a significant proliferative component of the disease. Repeated leukapheresis has been found to be ineffective for long-term management of advanced CLL, refractory to conventional treatment (25). In that study, 11 of 12 patients showed no improvement from anemia and thrombocytopenia, and had no reduction in symptoms.

Five patients with advanced Sézary syndrome, including one with lymph node involvement, were treated with lymphapheresis on 15 occasions. All patients were refractory to conventional therapy and had extensive symptomatic erythroderma. The diagnosis was confirmed histologically in all patients. One patient underwent lymphapheresis five times. Her lymphocyte and Sézary cell counts on peripheral blood in relationship to lymphapheresis are illustrated on figure 11. A decline in both counts followed treatment associated with a 60 percent improvement. In three patients, clinical improvements of 27 to 90 percent were observed following apheresis. The exact role of apheresis in the clinical improvement of these four patients is hard to assess since they received conventional treatment along with apheresis. One additional patient presenting with intensive pruritus, and in whom all therapy had been discontinued prior to treatment, reported a 50 percent improvement of skin lesions and complete remittence of pruritus following three lymphaphereses performed over 4 days. Figure 12 depicts results of apheresis on peripheral counts. In these cases, unlike a previously reported case (26), the peripheral leukocyte counts were normal. The average lymphocyte removal was 8.2 x 10^9 (range 2.3 to 17.8), whereas the average Sézary cell removal was 2.54 (range 0.24 to 6.05) 10^9, resulting in an average of 56 percent (range 17.4 to 85.0) reduction of original Sézary cell count. The average duration of the procedure was 3.07 hours (range 2.35 hours to 3.55 hours), and there were no reactions in this group of patients.

Figure 11.--Lymphapheresis



Each solid box indicates a lymphapheresis.

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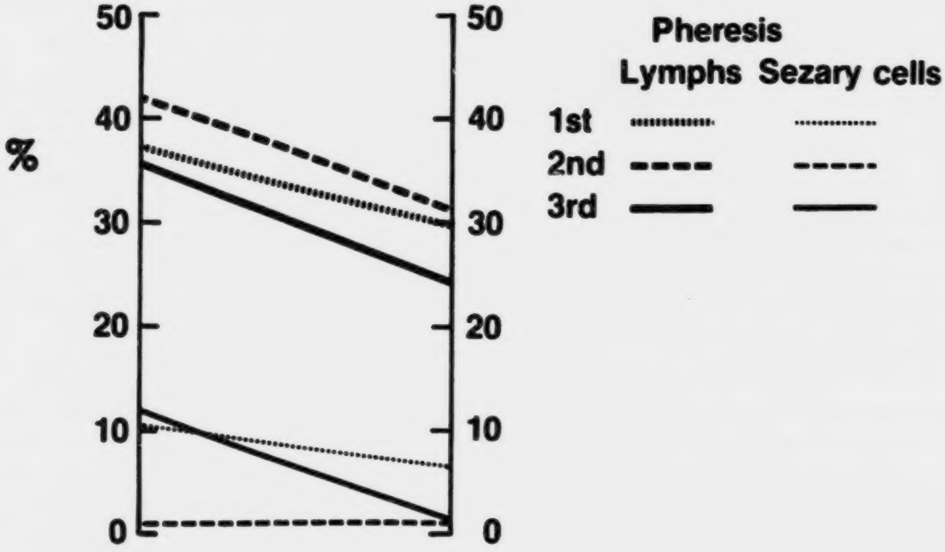
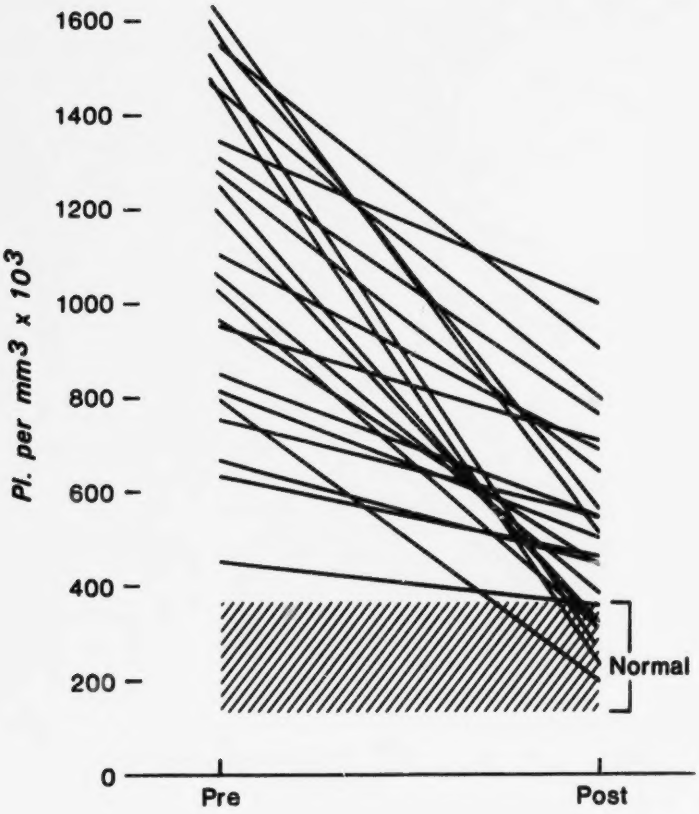
Figure 12.--Lymphapheresis in Sézary syndrome



PLATELETAPHERESIS

Therapeutic removal of platelets was performed 23 times on 14 patients with thrombocytosis. Thirteen patients had bleeding associated with the elevated platelet count and some had not responded to conventional therapeutic measures, including cytotoxic drugs and/or ^{32}P. The procedure was followed by cessation of bleeding, and the long-term control of basic disorder was achieved with the use of cytotoxic drugs or ^{32}P. One patient was pregnant, at term, and lowering of platelet count was done in preparation for induction of labor; one unit of blood was collected during each plateletapheresis for autologous use and as means of reducing red cell mass in this polycythemic and thrombocythemic patient. Five other patients had plateletapheresis done in an effort to lower counts and reduce attending complications prior to emergency surgical intervention. Surgery was successfully accomplished without complications. Overall 46 ± 15 percent reduction of platelet count was achieved per procedure in an average of 2.58 hours (range 1.30 hours to 3.55 hours) and with an average removal of 2.8 x 10^{12} (1.2 to 5.6) platelets. One patient experienced mild hypotension and two complained of nausea. The aim of therapy was to reduce the count as close to normal level as possible; some patients required two consecutive procedures to accomplish that. The lowering of platelet counts is, of course, temporary, and the long-term outcome depends on the use of conventional therapy. Pre- and postapheresis results in 12 patients are shown in figure 13. There were four reactions in this group of patients, three characterized by nausea and vomiting and one by mild hypotension. Otherwise, the procedure was tolerated well. Therapy of thrombocytosis with cytotoxic drugs or ^{32}P requires a minimum of 2 to 3 weeks before adequate reduction in platelet counts may be achieved, during which time patients remain at risk of hemorrhagic or thrombotic complications. Plateletapheresis by rapidly lowering peripheral counts has proven useful in the acute management of thrombocytosis in our experience as well as that of others (27).

Figure 13.--Therapeutic plateletapheresis



BLASTAPHERESIS

Leukapheresis resulting in the removal of large numbers of circulating blast forms (blastapheresis) was performed in 22 patients who suffered blast crisis of nonlymphocytic leukemia (NLL). Thirty-one procedures were performed with some patients having repeated procedures. Blast removal was performed on an emergency basis and in combination with skull irradiation and administration of hydroxyurea intravenously. The dangers from sudden death due to intracerebral hemorrhage in patients with acute NLL who have leukocyte counts of more than 200,000 are well known (29). The basic aim of the emergency treatment outlined above is the rapid control of leukostatic phenomenon. The additional utilization of apheresis in this group of patients gives further assurance that the peripheral blast population can be rapidly reduced to safe levels within the first 24 hours of therapy (28).

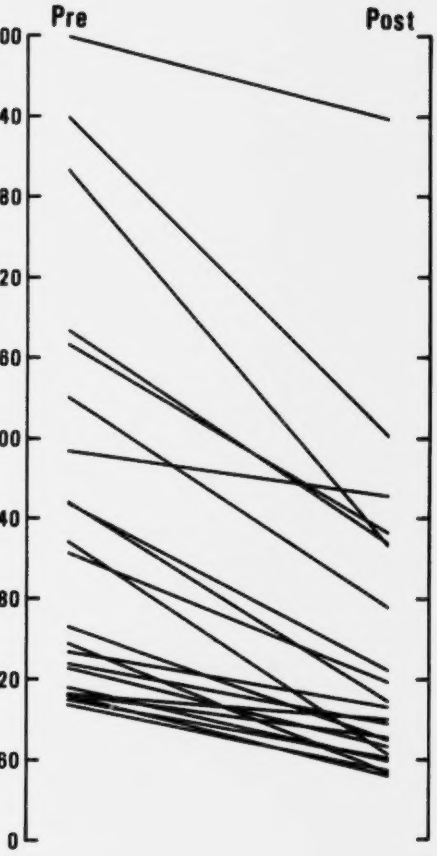
None of the patients treated in our group had documented evidence of intracerebral complications, and counts were controlled rapidly with combined therapy within 24 hours. Similar results have been reported by other groups (30,31). In a previous report (30) leukapheresis seemingly resulted in better survival rates when compared to a historical control group receiving identical chemotherapy but not leukapheresis.

In our experience, leukapheresis decreased total white cell count by 44 ± 15 percent of original count, and blast count by 39 ± 12 percent. The average leukocyte recovery was $3.8 \pm 1.9 \times 10^{11}$. The procedure lasted, on the average, 3.2 ± .3 hours. Pre- and postapheresis counts of 25 blastaphereses are shown in figure 14. We routinely employed a sedimenting agent: hydroxyethyl starch mixed with a citrated anticoagulant. Platelet loss caused by apheresis was on the average 15.4×10^{10} (range 1.1 to 65.0×10^{10}). In the majority of patients, the count started and remained above 20,000 platelets. This is important since the majority of those patients are severely thrombocytopenic, which could be aggravated by a careless removal of the platelet layer along with the white cell layer. The procedure was well tolerated despite the fact that the patients treated were very ill. With the exception of five episodes of mild hypotension associated with lightheadedness and nausea, no complications occurred during or immediately after apheresis.

CONCLUSION AND QUESTIONS

Like a number of new medical procedures, apheresis has gained rapid popularity and recognition. Performed on a trial-error basis and largely without the benefit of controlled studies, it has been and will continue to be applied to the treatment of a number of disease disorders with the purpose of evaluating its effects. Of necessity, the early trials were uncontrolled studies and have yielded data that merit confirmation and the backing of controlled studies to determine the real value of apheresis of the various kinds. Some controlled studies are already under way. We have begun the study of patients with scleroderma randomly assigned to two groups: The control group will undergo total plasma exchange in identical manner as the study group; the only difference will be that the control group will unknowingly receive their own plasma as replacement fluid. This information will remain unknown to the individual assessing objective and subjective clinical effect. Thus, we hope to evaluate the placebo effect associated with the physical attachment of the patient to an impressive, noisy apparatus—the cell separator.

Figure 14.--Therapeutic leukapheresis in blastic crisis of NLL $10^3/mm^3$



Questions still remain about the more physiologic and adequate replacement to use in TPE in the various conditions; although it may not be important in hyperviscosity or cryoglobulinemia, it certainly acquires relevance in immune-mediated diseases where defibrination, decomplementation and removal of mediator or inflammatory response may be of great significance to the underlying pathophysiology. Another factor is the cost and risk factor associated with the use of fresh frozen plasma or plasma derivatives very similar to it in composition (cryoprecipitate-poor plasma). One risk factor, hepatitis, is multiplied by the number of units from single donors required for a 5 liter plasma exchange (15 to 18 units). Another risk is that of life-threatening allergic reactions associated with anti-IgA antibodies. The disadvantages of fresh frozen plasma should be weighed against the potential beneficial effects of restoring naturally occurring immunosuppressive factors of potential importance for the control of autoimmune diseases as proposed by Gilcher (32). Designing more efficient techniques of platelet sparing leukapheresis appears more important than leukocyte sparing in plateletapheresis. The questions of the safety of the poorly excreted hydroxyethyl starch and its potential for allergic reactions and dermatologic complications need to be elucidated, as does the use of other sedimenting agents.

The infrequency and rarity of some of the diseases where apheresis is being employed underscore the need for cooperative studies, which could expedite data gathering and publication of scientifically sound results. Finally, a word of caution is in order: apheresis is an expensive and potentially hazardous procedure that should not be employed indiscriminately and should be carried out in optimal conditions and under close medical supervision by nurses or technicians duly trained and familiar with patient care.

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DISCUSSION

Dr. Wolf W. Zuelzer: I have asked Dr. Pineda to stay during the discussion. His paper is now open for discussion.

Dr. Curt A. Ries: It was very interesting to hear your data. I would like to ask you [about] a few points that don't pertain directly to your results so much as to the procedural matters. How do you work vis-a-vis your human experimentation committee? Or have you decided that this is a treatment modality that does not require approval by human experimentation? How much do you charge for a procedure, or is it done under a grant?

Is it the blood bank that supervises the procedure, and if so, how do you deal with the problem of getting clinicians involved, since these are often very sick patients who require a lot of close monitoring? Do you hire clinicians in the blood bank to supervise the nurses and take care of these patients, or do you have the medical service do that?

Dr. Pineda: Thank you for the...questions. They give me the opportunity to give credit to a very dedicated group of people--my apheresis technicians. There are five sitting in the back of the room, and they deserve a lot of praise. We have five technicians in our team, and the medical supervision is given by the blood bank, when the procedures are done in the blood bank, and that would be for patients who are not critically ill, obviously.

For the patient who is critically ill, we take our machines to the hospital and in that case, the primary service provides the medical coverage. We do not require additional medical personnel for either the hospital or the blood bank procedures. We do have residents in the blood bank, as well as the staff consultants, who provide the medical coverage.

There is always a physician present when there is a procedure, either therapeutic or when we are collecting granulocytes or platelets [from a donor]....

To answer your first question: Some of the work you just saw is research. It has been approved by our institutional human studies committee and is being funded by grants, mostly money coming from intramural funds.

We have had human [studies and] research committee approval for all the procedures that we have done.

Dr. Ries: The cost?

Dr. Pineda: The expensive part, of course, in total plasma exchange is the replacement. The replacement of 5 liters with normal serum albumin costs anywhere from $400 to $500 per procedure....

The cost of the run also depends on the type of machine we use. We use the semicontinuous flow centrifuge for the majority of procedures... because it is less expensive to run. In that case there is a flat charge close to $300 per procedure. When we use the continuous flow centrifuge, the charges are higher. For the therapeutic procedures thought to be well-established procedures, we have charged the patient or third party payors....

Dr. Zuelzer: In the interests of fairness and time, I am going to ration the questions to one per discussant.

Dr. Janet Cuttner: I was very impressed by your extraordinarily good results, and I just would like to caution the audience not to expect these great results in all these diseases. Thrombotic thrombocytopenic purpura happens to be a disease that I am personally greatly interested in; we have done two plasma exchanges, and there has been absolutely no response to it, and we have had to go on to other treatments.

Dr. Bruce McLeod: Would you specify what was the replacement fluid you gave the patient with TTP?

Dr. Pineda: The replacement fluid for the first seven exchanges was 5 percent normal serum albumin, and for the last two, fresh frozen plasma. I had the opportunity to talk by phone with Dr. Gilcher, who has treated 14 patients with TTP, and he suggested that fresh frozen plasma was a better replacement for TTP. Dr. Gilcher, you might want to comment on that.

Dr. McLeod: Before Dr. Gilcher describes his experience, let me mention that we have had two patients with TTP who were treated with plasma exchange and received fresh frozen plasma as replacement fluid. Both seemed to respond dramatically with improvement in hematologic parameters.

Dr. Dennis Goldfinger: One of the areas of cytapheresis, Dr. Pineda, that you did not mention, and which is not included in the program, is red cell exchange apheresis. We have recently had rather spectacular success in exchanging three patients with sickle cell disease.

The first patient had severe unremitting priapism; the second patient, an adult, had respiratory compromise with severe hypoxemia; and most recently, just 2 weeks ago, a young 60-pound boy, with severe respiratory compromise, was treated, using the new Haemonetics pediatric bowl, which allows one to apply this kind of technology to kids.

It is well known that exchange transfusion in sickle cell disease is beneficial. If sickle cells are the problem, then removal of those sickle cells is likely to be beneficial.

However, one aspect of apheresis which is quite important is the availability of an apheresis team.... When I was a house officer at Bellevue, we had to do our own CBC's; we did very few white counts, very few CBC's; our patients had to be nearly dead before we would do this, because we did it ourselves.

This is the argument we used for this most recent kid; and the pediatricians jumped at the idea because they did not have to do it themselves. The ability now for a physician to write an order to perform an exchange apheresis will make this available.

This has been a spectacular success in these three patients: instant recovery from the complications.

Dr. Pineda: There has been a report of a red cell exchange in a patient with PNH, that was reported at the last AABB meeting.

Dr. Ronald Gilcher: We have actually treated 15 patients with TTP, but not all by plasma exchange.

We began treatment in 1971 using whole blood exchange. We treated seven patients with whole blood exchange...five survivors. In early 1976, we began treatment of TTP using plasma exchange [with] the Haemonetics Model 30 cell separator as the machine for the exchange. We have six of eight survivors out of that group. Our exchange media has been two-thirds fresh frozen plasma and one-third 5 percent albumin....

But we feel that plasma exchange is, at the present time, combined with antiplatelet therapy plus corticosteroids, the optimum treatment of choice for TTP.

Dr. Pineda: I forgot to mention that the three patients we described were treated with antiplatelet therapy, as well as the steroids, in addition to the exchange.

Dr. Zuelzer: Any other comments or questions for Dr. Pineda? Thank you, Dr. Pineda....

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LEUKAPHERESIS IN ACUTE MYELOBLASTIC LEUKEMIA

Janet Cuttner, M.D.
Richard J. Meyer, M.D.
Edward P. Ambinder, M.D.
Michael L. Greenberg, M.D.
Gail Button, R.N.
James F. Holland, M.D.
Mount Sinai School of Medicine
New York, New York

Patients with acute myeloblastic leukemia who present with marked elevation of the WBC (over 100,000/µl) have a high mortality (5,6,11,16, 18). This is due to leukostasis, especially in the brain and the lungs, with subsequent bleeding. In 1960, Freireich et al. reported on autopsy findings in 81 patients with acute leukemia (5). Eighteen (22%) died of CNS bleeding and one-half of this group had a white blood count greater than 300,000/µl. Pathologically, the lesions were confined to the white matter. Microscopically, the intracerebral lesions consisted of masses of leukemic leukocytes surrounded by fresh hemorrhage. They described another lesion, which they called "leukostasis," which consisted of slightly dilated, thin-walled, intracerebral vessels that were filled with an almost pure collection of leukemic cells. Leukostasis was not found in patients with leukocyte counts below 100,000/µl. These findings have been reaffirmed by others (11). It is now recognized that the lung and the brain are the two organs most importantly involved in leukostasis. McKee and Collins reported on clinical and autopsy data on 206 cases of leukemia seen from 1956 through 1971 (18). They found intravascular leukemic aggregates or thrombi in all patients with AML or CML with WBC greater than 200,000/µl, and in 11 of 24 patients with counts between 50,000/µl and 200,000/µl. No patients with counts below 50,000/µl had frequent thrombi and the frequency of aggregates was also much less. Leukemic aggregates have also been reported to cause myocardial infarction (23). Patients with very elevated white cell counts commonly present with hyperuricemia and, if treatment is not initiated quickly, urate nephropathy and renal failure may ensue (17). These factors may account for the higher early mortality reported in patients with AML presenting with blast counts exceeding 50,000 (13). A method of rapid and early lowering of white counts may decrease this mortality (7).

Extracorporeal irradiation of blood has been used by Schiffer to control white counts in AML for from 13 to 97 weeks (24). Cytapheresis has been shown to rapidly lower white blood cell counts in a CML patient presenting with papilledema, leading to neurologic improvement (26).

Eisenstaedt reports the rapid lowering of white blood counts in a patient refractory to chemotherapy using the Haemonetics Model 30 processor (3). We have supported a 15-year-old pregnant patient solely with leukapheresis and red cell transfusions for the 13 weeks necessary to successfully complete her pregnancy (19). She remained well for this period with reduction in peripheral white counts from 65,000/μl to 4,800/μl, improvement in platelet count from 54,000/μl to 154,000/μl, and a decrease in gingival hypertrophy.

Leukapheresis has been reported to increase the fraction of leukemic marrow blast cells in DNA synthesis in four of seven patients as well as increasing the fraction of mitoses in four of five (10,14,22). Despite theoretical advantages to increasing cells in DNA synthesis, no advantage was associated with leukapheresis in these patients (9,22).

Leukapheresis has been available at the Mount Sinai Hospital (MSH) since December 1974. It was initially used as a means of collecting leukemic blasts for an immunotherapy program in AML. We then began a pilot program of therapeutic leukaphereses in patients with AML presenting with a WBC of 100,000/μl or higher. Nine consecutive patients with acute myeloid leukemia and peripheral counts greater than 100,000/μl were leukapheresed for from one to three times (table 1) prior to chemotherapy with daunorubicin 45 mg/m^2/day x 3 and cytosine arabinoside 100 mg/m^2/day x 7. Initial white blood counts ranged from 105,000/μl to 280,000/μl. During leukapheresis, a total of 4.6×10^{11} to 2.3×10^{12} ($\bar{x}$ 7.3×10^{11}) WBC were removed, lowering the WBC from 49,000/μl to 186,000/μl, a mean decrease of 50 percent. Of primary importance was the fact that seven of the nine patients entered a complete remission, one a partial remission, and one died on day 4 of central nervous system bleeding (20). In comparison, of 11 consecutive patients with AML and WBC >100,000/μl seen in 1974-1976, just prior to the above patients, and receiving identical chemotherapy but not leukapheresis, four entered complete remission, one partial remission, and six died (table 2). This represented a 36 percent complete remission rate, compared to 78 percent for the group undergoing leukapheresis. Comparing the complete remission rate of these two groups, the chi-square, one degree of freedom, is 3.4, $0.05 < P < 0.10$. Of corollary interest was the fact that leukapheresis resulted in an apparent increased fraction of cells in DNA synthesis, perhaps explaining these disparate results (9).

Because of the promising results of the pilot study, a randomized controlled study of leukapheresis in AML was undertaken in July 1977.

METHODS AND MATERIALS

All newly diagnosed patients with AML who are 13 years of age or older and who present with a WBC of 50,000/μl or above are eligible for study.

Table 1.--Pilot study: Induction results in patients with AML with initial WBC > 100,000/μl

			Therapeutic Leukapheresis					
			Number of Days	WBC/μl x 10^3			Remission	Day M_1
Patient	Age	Diagnosis	Leukapheresis	Pre	Post	Total Yield	Status	Marrow
PG	35	AML	2	105	55	3.1×10^{11}	CR	26
EM	71	AMML	3	124	49.9	4.6×10^{11}	CR	29
PW	32	AML	3	137	82	1.1×10^{12}	CR	34
DC	24	AML	2	159	93.8	6.4×10^{11}	CR	27
JW	65	AMML	3	175	52	9.0×10^{11}	PR	71
SA	53	AML	2	190	---	8.8×10^{11}	Died D4 CNS	--
JN	56	AMML	2	204	116	1.1×10^{12}	CR	26
LF	17	AML	1	278	178	6.0×10^{11}	CR	38
DR	17	AML	2	280	186	1.8×10^{12}	CR	22

phereses. There is no randomization to zero leukapheresis because of the necessity to provide cells for our immunotherapy program. Prior to and after completion of leukapheresis, bone marrow and peripheral blood specimens are drawn for routine studies (appendix 1). In addition, specimens for labeling and mitotic indices are collected.

Table 2.--Pilot study: Induction results in patients with AML with initial WBC >100,000/μl without therapeutic leukapheresis

Patient	Age	Diagnosis	Initial WBC/μl	Remission Status	Day M_1 Marrow
AC	50	AML	100	NR	--
MK	32	AML	109	CR	32
AB	6	AMML	113	NR	--
AO	84	AMML	133	NR	--
AK	56	AML	225	CR	58
EC	53	AML	253	NR	--
BB	50	AML	254	CR	60
LG	75	AML	273	NR	--
FN	63	AMML	277	NR	--
JV	53	AML	158	PR	--
JD	43	AMML	205	CR	--

Leukapheresis is performed using the Haemonetics Model 30 blood processor with either a two-arm or, when vein access was difficult, a one-arm technique. Anticoagulation consisted of ACD Formula A. For all donors the 225 ml disposable bowl was used. Harvesting of leukemic blasts occurred at a flow rate of 20 ml/minute just after the buffy white platelet layer was removed. Collection is continued for removal of the entire buffy coat layer whose depth is dependent upon the white blood cell counts (usually ∿ 150 cc). At the completion of each cycle, the red blood cells and plasma are reinfused. Six cycles, each lasting 20 minutes, are performed during each day's procedure. Red blood cell transfusions are given as needed to keep the hemoglobin level above 10.0 g/dl. Platelet transfusions are given as needed to keep the platelet counts above 25,000/μl.

Twenty-four hours after the last leukapheresis, all patients are treated with daunorubicin 45 mg/m^2 as a single intravenous injection on days 1, 2, and 3, and cytosine arabinoside 100 mg/m^2 in a continuous infusion on days 1 through 7. Definition of complete remission is according to CALGB criteria.

Serial marrow and/or blood specimens were obtained for LI (labeling index, i.e., percentage of cells in DNA synthesis or labeled by tritiated thymidine) and MI (mitotic index, i.e., percentage in mitosis) (9). Autoradiographs were prepared after a 1-hour incubation with tritiated thymidine. LI was determined by counting at least 1,000 leukemic cells per specimen. MI was the percentage of mitoses per 10,000 leukemic cells per specimen.

Statistical analysis was done using the Student's t test and the correlation coefficient based on a linear regression.

RESULTS

As of March 1, 1979, 17 patients had been entered on the study. The ages ranged from 16 to 74. Initial WBC's have ranged from 67,000/μl to 263,000/μl.

Ten patients were randomized to receive three leukaphereses, and seven to receive one leukapheresis. Three patients were excluded: two in the three-leukaphereses group, one in the one-leukapheresis group; two for private M.D. violations of chemotherapy protocol and one for patient refusal to undergo prescribed chemotherapy. One patient is currently undergoing chemotherapy and is too early to evaluate. No patient manifested adverse effects of leukapheresis and all completed their prescribed cytapheresis course. One patient in the one-leukapheresis group died on day 2 of chemotherapy of progressive CNS bleeding; one on day 12 of overwhelming infection. One patient in the three-leukaphereses group died on day 6 of sepsis. One patient is too early to evaluate.

Of the 13 evaluable patients, 8 (61%) obtained M-1 marrow status. Of these, three received one leukapheresis, and five received three leukaphereses (table 3). Of the 13 evaluable patients, 9 had a WBC >100,000/μl. Of these nine patients, six (66%) obtained M-1 marrow status. Two of four patients with one leukapheresis (50%) and four of five patients

Table 3.—Results of therapeutic leukaphereses plus ARA-C + DNR

	Number Entered	Number Evaluated	Too Early	Number Disqualified	Number M-1	Percentage M-1	Number N.R.
1 Leukapheresis	7	6	-	1	3	50	3
3 Leukaphereses	10	7	1	2	5	71	2
	—	-	-	-	-	—	-
Totals	17	13	1	3	8	61	5

(80%) receiving three leukaphereses obtained M-1 marrow status. This difference is not significant at the present time.

The comparison of WBC yields in the patients who achieved a complete remission compared to those who failed to respond can be seen in tables 4 and 5. The WBC's before leukaphereses were similar in both groups. All patients who had three leukaphereses had their WBC decreased to less than 100,000/µl.

The average percentage of decrease in WBC for patients undergoing three leukaphereses was 51.4, compared to 16.9 percent for those patients who had one leukapheresis. The adjusted t value for this difference was 2.89.* This is significant at $P = 0.05$.

The results of the leukokinetic studies (table 6) compared the group of patients who achieved an M-1 marrow with the group that did not do so for the following parameters:

- initial LI, maximal LI, ratio of maximal to initial LI, and the ratio of 24-hour to initial LI for marrow and blood specimens separately;
- initial MI, 24-hour MI, and the ratio of the two;
- initial WBC, WBC after all leukaphereses, and the ratio of the two; and
- total cells leukapheresed.

In no case was there a significant difference between the two groups.

Correlation coefficients were determined for the following pairs of parameters for marrow and blood specimens separately:

- ratio of maximal LI to initial LI or ratio of 24-hour LI to initial LI; and
- initial LI, yield, or ratio of final WBC to initial WBC.

---

* 2.9 >2.5, which is the critical value for Cochran's approximation of t's of unequal variances (25).

Table 4.--Patients who obtained an M-1 marrow status

Patient	Number of Leukaphereses	White Blood Count/µl Pre	/	Post	Decrease White Blood Count/µl	Percentage Decrease	Yield x 10^{11} White Blood Count
		Leukapheresis					
AF	3	263.5	/	85.5	178	68	5.2
JS	3	82	/	42	40	48	11.2
SK	1	67	/	43.2	23.8	35	1.6
FN	3	126.5	/	52.5	74	58	9.3
GK	1	151	/	163	+ 12	+ 7.9	2.7
TR	1	143	/	60.8	82.2	57	1.3
AS	3	150	/	63	87	57	6.4
AR	3	152.5	/	9.5	57.5	37	4.0
Mean		141.9	/	64.9	69.3	46	5.2

Table 5.--Patients who failed to enter M-1 marrow status

Patient	Number of Leukaphereses	White Blood Count/µl Pre / Post Leukapheresis	Decrease White Blood Count/µl	Percentage Decrease	Total Yield x 10^{11} White Blood Count
GF	1	173.5 / 175	+ 1.5	+ .08	4.3
PN	3	81.8 / 53	18.8	23	1.8
RH	3	114 / 34.3	79.7	69	2.7
MH	1	204 / 201	3	1.5	5.2
RK	1	64 / 53.1	10.9	16	.8
Mean		127.5 / 103.3	23	21.9	3.0

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Table 6.--Cellular kinetic studies

				Bone Marrow				Bone Marrow				Peripheral Blood		
Patient	Number of Leukapheresees	Result of Therapy	Pre* LI	24* LI	End* LI	Post* LI	Pre MI	24 MI	End MI	Post MI	Pre LI	24 LI	End LI	Post LI
AF	3	M-1	11.0	15.7			0.12	0.4			5.8	4.3		
JS	3	M-1	20.0	26.7	25.4		0.3	0.4	0.2		0.8	1.2	1.6	
AS	3	M-1	18.6	19.1		18.0	0.17	0.14		0.54	10.1	11.0		12.0
SK	1	M-1	--	--		--	--	--			7.5	5.1		5.1
GK	1	M-1	--	--		--	--	--			8.0	6.5		6.5
RH	3	N.R.	15.9	21.2		24.4	0.22	0.23		0.12	2.0	1.3		0.4
RN	3	N.R.	81.8	53			--	--			1.0	1.8		
GF	1	N.R.	18.5	13.0		13.0	0.5	0.1		0.1	5.5	2.5		2.5
MH	1	N.R.	16.5	19.4		19.4	0.4	0.5		0.5				

LI = Labeling Index

MI = Mitotic Index

Pre* = Preleukapheresis

N.R. = No response

24* = 24 hours after first leukapheresis

End* = Immediately after one or three leukaphereses

Post* = 24 hours after last leukapheresis and before chemotherapy

The only suggestions of reasonable correlations seen were the increasing fractional rise in LI at maximum and at 24 hours in correlation with the increasing fractional decrements in the WBC after leukaphereses. In both cases, the correlation coefficient (r value) was approximately 0.70, but the P value for the t statistic of each correlation was only 0.1 in each case.

DISCUSSION

Cytapheresis techniques have been applied with varying success to the treatment of chronic myelocytic and lymphocytic leukemias, the Sézary syndrome, and thrombocythemia (1,2,4,8,12,27,28,29). The safety of leukapheresis in AML was proven in early procurement programs for immunotherapy (15, 21).

The leukokinetic studies generally confirm and extend previous observations made in similar patients by other observers (9,14). Five of six patients had an increase in the fraction of marrow cells in DNA synthesis, demonstrating the responsiveness of these replicating leukemic cells to a stimulus associated with leukapheresis. Although four of six patients also had increments in MI of marrow leukemic cells at 24 hours, the values were all less than 1 percent and are within the statistical error of counting at that level. LI shows no consistent pattern in the peripheral blood, but that is not surprising since these are probably cells in transit and not an important site of replication. There was no correlation with any of the parameters measured with the results of therapy.

Patients with very elevated white cell counts are at great risk of dying from the complications of leukostasis, especially in the central nervous system and the lungs. This same group of patients commonly presents with hyperuricemia, and therefore, definitive treatment, which is of critical importance, may have to be delayed while the patient receives allopurinol and alkalinization. Pretreating patients with cranial irradiation does not prevent the complications. Leukapheresis is a safe procedure which can "buy time" until definitive chemotherapy can be given. Our pilot study of nine AML patients with WBC >100,000/µl, who received one, two, or three leukaphereses, confirmed the safety of the procedure. Seven of nine patients entered a complete remission. We are currently randomizing patients to one or three leukaphereses. Our early results show that four of five patients with a WBC of 100,000/ µl or greater who received three leukaphereses enter complete remission, as compared to two of four who received one leukapheresis. There is a much greater decrease in WBC yield postleukapheresis in the responders. This decrease in leukemia cell body burden may permit chemotherapy to be more effective. Five of six patients who presented with a WBC >100,000/µl had their WBC decreased to below 100,000 by leukapheresis alone. All patients undergoing three leukaphereses had their WBC lowered to below 100,000/µl. This, in and of itself, may decrease the complications of leukostasis allowing definitive chemotherapy to work. In contrast, two of three patients who presented with WBC > 100,000/µl and who had virtually no decrease in WBC after leukapheresis did not respond to chemotherapy and died.

If these early results continue to show the same trend, then leukapheresis would be indicated in all patients with AML presenting with a WBC greater than 100,000/µl.

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APPENDIX 1

LABORATORY DATA

Study	Initial	Daily During Leukapheresis	Daily All Patients	3/Week	1/Week
Hematologic					
CBC, PLTS, RETIC's	X	before and after leukapheresis	X		
Haptoglobin	X				X
Lysozyme-Serum	X	X			X
Protime, PTT, Fibrinogen	X	X			X
Fibrin split products	X	X			
Bone marrow with ^{3}HTDR + MIOTIC indices	X	X			
UB BC-TC II 12	X	X			X
ABO typing	X				
HAA	X				
Chemistry					
SMA 6, 12	X	X		X	
Protein Electrophoresis	X				
Immunoelectrophoresis	X				
Chest X-Ray	X				at the end of last leukapheresis
EKG	X				

DISCUSSION

Dr. Zuelzer: Dr. Cuttner's paper is now open for discussion.

Dr. Harry Taylor: I didn't hear you mention the use of a red cell sedimenting agent in your protocol.

Dr. Cuttner: We just used ACD formula.

Dr. Taylor: You did not use HES?

Dr. Cuttner: No.

...

Dr. Taylor: Do you suppose if you had used a red cell sedimenting agent, you would have gotten greater increments--greater yields?

Dr. Cuttner: I'm sorry.... Did you say, did we use an agent? ...yes, we did....

Dr. Taylor: I was asking whether or not you used HES--hydroxyethyl starch--for red cell sedimentation.

Dr. Cuttner: No, we do not use HES. I am sorry. I misunderstood you.

Dr. Amanullah Khan: I just need to clarify a couple of points on the remission rates. You gave two or three different remission rates, and I was kind of confused on that. One was 34 percent remission rate in the prior AML patients that were treated in the Institute. Then you mentioned a 67 percent remission rate on the overall, I guess, AML; and then you mentioned a 72 percent remission rate and a 78 percent remission rate in the treated patients. Could you clarify for me what is the overall remission rate? If it is 67 percent, then it is not any different from your 60 percent or 72 percent with leukapheresis; it seems [that] virtually there is no difference. Could you explain that?

Dr. Cuttner: Yes. I presented two studies. The first was the pilot study, in which we leukapheresed nine patients. Seven of nine patients entered a complete remission. We compared them with the patients just prior, who received identical hemotherapy, and that is where the remission rate--it was 4 out of 11, for 39 percent. That was the pilot study.

Then I went on to the randomized study that we are now doing, where patients are receiving one or three leukaphereses. And it is in that study that the overall remission rate is 61 percent.

Dr. Khan: So if you compare the patients with leukapheresis, what would be your comparable group? Will it be the remission rate of 67 percent?

Dr. Cuttner: No. You see...in the pilot study we compared patients who received leukapheresis with patients who did not. In the current study, though ideally we should compare it with no leukapheresis, we can't, because we have an immunotherapy program. So we are comparing three [leukapheresis procedures] versus one.... The remission rate for patients who only got one leukapheresis is 50 percent, compared to 71 percent for patients undergoing three leukaphereses.

Dr. Khan: Will you do sham leukapheresis in the AML?

Dr. Cuttner: I would not. No.

Dr. Edwin Taft: Could you elaborate on the rationale for a 3-day protocol of leukapheresis?

Dr. Cuttner: Yes. We originally used it in the pilot study, when we had two or three patients with severe hyperuricemia--let's say uric acid of 17 or 18--where we leukapheresed the patient for 3 days while we were giving alluropurinol or alkalyzation in an effort to get the uric acid down. But we were very impressed with how well we could get the white blood count down, and that is the reason for choosing three. Now, these are very small numbers, that's for sure; but thus far, [with] the patients who have undergone three leukaphereses, we have been able to lower their white counts in all cases [to] below 100,000.

Dr. Taft: We have used a 1-day protocol, because our own retrospective control series shows an 80 percent mortality in the first 4 days. We have chosen to attempt to get the white count under 100,000, and we think 100,000 is probably the critical level.

Our own experience also suggests that the blast population of acute leukemics is anything but homogeneous; that some blasts will separate out at relatively easy conditions, close to those optimum for lymphocytes, but others do, in fact, require HES.

Now, the other thing we have seen--our end results in a smaller series than yours are not quite so good. We are observing a late mortality, predominantly pulmonary mortality, 3 to 4 weeks into induction with a high complication rate of pulmonary hemorrhage and pneumonia. We think that there may be damage prior to the time that we get these patients. We think that we need additional data as far as the pathophysiology of this whole syndrome is concerned.

Dr. Cuttner: I would agree with you that there is a difference. The two patients who did not respond, who underwent leukapheresis--we were unable to get their white counts down to less than 100,000, so there does appear to be a difference. But in the majority of cases, we have been able to [lower the counts]. We have not seen a pulmonary complication per se that we could not relate to [the disease]. We do support the patients strongly; we have a big white cell program, as well as a platelet program.

Dr. Larry Grossman: As you mention, your numbers are small, both in the retrospective groups that you have looked at for us and in the control group that you now have under way.

I wonder if you could try to elucidate for us some of the causes of death or failure to respond in the patients that have not been leukapheresed, and try to relate them to things that you may be correcting by leukapheresis.

Dr. Cuttner: The majority of patients in the pilot study that did not undergo leukapheresis died of mainly CNS hemorrhage, but a few of them died of pulmonary complications. We didn't get autopsies on all of them. We presume it is related to the leukostasis, but I can't be entirely certain. They died very quickly, which is what is in the literature, within about 3 or 4 days.

Dr. Grossman: Was there any difference, though, in the platelet count, for example, in that group of patients, as opposed to the ones that were leukapheresed?

Dr. Cuttner: I don't think so.

Dr. Winston Ho: We have treated in the past several years about 56 to 60 patients, using a three-drug regimen similar to yours; and they have been patients with leukemia. And we only occasionally do leukapheresis in patients who have white counts of perhaps 70,000 to 100,000 for the purpose of procuring cells for immunotherapy studies.

Our overall remission rate, using that regimen, is approaching 86 percent, and we have made an analysis of the patients who have had leukapheresis and [of the] others, and find no difference....

I would like to ask a question, in regards to what you think is the significance of... the peripheral white count [being] reduced by 50 percent; in some patients who have got a count of 100,000, and others who have got a count of perhaps 60,000, [for whom] you have done the leukapheresis, do you think that the overall leukemic burden, the low number, has really fallen that much, to make the difference in your remission rate?

We have the feeling that the success of remission depends on the support system that one has in order to maintain the patients through the intensive chemotherapy.

Dr. Cuttner: I will try to answer all those questions. There is no doubt that support is vital to the treatment of acute myeloblastic leukemia; I would agree with you. However, our feeling is that in patients with a white count over 100,000, there is an advantage to leukapheresis, because those are the patients that are going to die of leukostasis before chemotherapy is going to be able to work.

Now, if you have a remission rate of 87 percent, that is obviously extraordinary. But I would say that most people don't have that remission rate. I am a member of a very large group which Mayo Clinic happens also to be a member of, the Cancer and Leukemia Group. In that group, the remission rate for AML is about 55 percent. I think we have to put things in that kind of possible perspective.

Dr. Jay Menitove: Have you treated any of the patients with hydroxyurea and cranial irradiation? You have a 3-day period, and I assume that would work before the 3 days; and I wonder if leukapheresis is indeed necessary all of the time.

Dr. Cuttner: None of the patients received any other treatment outside of support and leukapheresis. My own personal luck with hydroxyurea is not good.

Dr. Alvaro Pineda: In our patients, although we did not see intracranial complications following the leukapheresis, the survival... has been very poor, Dr. Cuttner; and, in fact, we questioned whether the age of the patients might not be the reason. Our patients have been either 50 or older, and I wonder if your group is younger than ours, and that would be one reason why ours aren't surviving, and yours are.

Dr. Cuttner: Well, our oldest patient, I think, was 74 or something like that. We have, as you know, the Haemonetics machine, which can be brought up to the floor, and almost all of our patients are leukapheresed right on the floor. We don't bring them down to the blood bank to leukapherese them.

We do treat, I would say, up to [age] 75 with the same chemotherapy irrespective of age.... We have found it really depends on the condition, not the actual age of the patient.

Dr. Gilcher: Some comments about hydroxyethyl starch. We have looked at leukapheresis with and without hydroxyethyl starch and what we found is that the differential of the cells removed in acute leukemia is predominantly blasts when you do not use hydroxyethyl starch. So we were truly doing more of a blastapheresis.

We have also looked at CML, and there it is very clear that the cells apheresed off without using hydroxyethyl starch must be of a younger cell population; and predominantly the younger cells, the blasts, are removed. So we have been doing our blastaphereses without hydroxyethyl starch in order to conserve the more mature cells within the patient which were removed when we did use hydroxyethyl starch.

Dr. McLeod: I have a purely technical question. When I have used the Haemonetics instrument to do therapeutic leukapheresis, I have had difficulty judging when to discontinue the collection phase. Do you have a protocol for therapeutic leukapheresis which specifies flow rates, collection times, and visual clues for stopping the leukocyte collection phase?

Dr. Cuttner: Well, we do--we use a flow rate of 20 ml per minute; and we do six cycles in a length of about 20 minutes each. And we do this more or less in all of our patients.

Dr. Neil Blumberg: One comment that is germane to Dr. Pineda, and to perhaps a lot of other people here, is the problem of thrombocytopenia and decreasing platelet counts in people undergoing therapeutic leukapheresis, [which] might be possibly addressed by something that people at our center have been working on. It is possible, after the procedure, to separate the leukocytes and red cells from the residual platelets with very high efficiency. This was in an abstract that is published in Transfusion by Dr. Katz and his colleagues. You could actually give the platelets back, if you are having trouble separating them during the procedure.

The second thing is, I am confused by your experimental design. To me, it seems that two things are going on here: One, a short-term mortality you are looking at, due to leukostasis, and the endpoint there would presumably be survival for some short period of time to enable the chemotherapeutic agents to yield a remission. The second thing is whether leukapheresis, mild or intensive, might have some effect on the ultimate rate of remission. And those are really, to me at least, two separate issues. One possibility is that if you did intensive leukapheresis, that you might be reducing the overall tumor burden, which is known to correlate to some extent with the remission rate.

I was wondering if you had any thoughts on that, and specifically, I was wondering if anybody had any data on the extent to which the circulating cells represent [a] percentage of the tumor burden in acute leukemias.

Dr. Cuttner: Well, I think the principle is twofold. One is to prevent the effects of leukostasis, and allow the chemotherapy to work. This is for patients with very high white counts. The other is, does leukapheresis change the cellular leukokinetics, allowing chemotherapy to work?

I showed one slide on our kinetic data. Again, it is only about 9 or 10 patients; we did see some changes. The changes that we saw were not significant. However, I think the numbers are too small to say anything, at the present time.

Dr. Khan: Again, we come back to the problem of using controls. It is very desirable, of course, nobody will deny, to have controlled trials. But...you know we may get carried away with the controls business.

I would like to narrate a brief story I heard once, in a meeting. There was a physician who had a very good friend who had lesions on both of his arms from some unknown malady. He thought that there was a treatment he would like to use, and so he convinced his friend that, "I have this treatment I would like to apply to one arm, and keep your other arm as a control." The friend went along. They usually shared various things together, and one day they were on the beach, swimming, and the friend who had the disease got engulfed by a shark. The physician, of course, was affected by the tragedy; but when he came back, being a scientist, he decided to write some notes about what happened with his treatment, in his book. And he wrote the following: "The patient was eaten by a shark. However, the control arm disappeared first; and the treated arm lasted longer."

So, I think we have to keep in mind that these things can happen when you do extremely controlled studies, and especially...sham procedures....

Dr. Zuelzer: I don't know whether Dr. Cuttner wishes to respond to the shark story, but, listening to the paper and to the discussion, I am surprised that no one raised a more fundamental question which, since we do have time, I will take the chairman's privilege to bring up. And that is, it seems to me that everybody here accepts the leukostasis theory as the "gospel." Is there no one who questions that? Is that accepted?

You see, if you take what you see under the microscope in the brain of these patients, and try to correlate it with clinical, at least in children, which is something I am familiar with, there is no correlation. And if you see chronic myelogenous leukemia with a white count for long periods of time which is very, very much higher, it becomes difficult to see why a white count of 50,000 or between 50,000 and 100,000, with perhaps a red cell mass of several million, should have a clinical significance. That is why I would like to ask Dr. Cuttner why she chose 50,000 as the starting point in this treatment if, in fact, leukostasis is considered the ultimate indication here.

Dr. Cuttner: Well, 50,000 was chosen empirically. I really think in retrospect that 100,000 is the best choice. But to get back to what you said about CML, leukostasis is seen in patients, and has been reported in several studies in patients with white counts over 100,000. But these are in patients with acute leukemia, and these are blasts. As we saw in the lung biopsy, patients with CML, as we all know, have very high white counts, but they don't have blasts and they don't have this manifestation until they become blastic.

We have [seen] patients with CML enter the so-called blastic or accelerated phase of their disease; indeed they can develop leukostasis, and they do develop a central nervous system bleeding. Similarly, patients with chronic lymphocytic leukemia--in which we also have a leukapheresis program--can have white counts of 200,000 to 300,000, and they virtually never have signs of leukostasis. But I think it has to do with the blasts; the blasts are sticky....

Dr. Zuelzer: I may have opened a Pandora's box or a can of worms here, but we still have a few minutes' time.

Dr. Ries: Our experience in the treatment of acute leukemia at the University of California [San Francisco] is that we don't lose patients early because of high white counts anymore. I think with aggressive chemotherapy, using two or three drugs and generous daunomycin, within 3 days, even without leukapheresis, the counts are well under control.

Our high-count patients do poorly in the long run, however, probably because they have a heavy tumor burden on board. Their complete remission rate tends not to be nearly as high as in the lower tumor burden group.... The gentleman that commented on the two different aspects of Dr. Cuttner's data, I think, made an important point there. In terms of treating the leukostasis--I don't know whether it exists or not. We don't really see that much of a problem anymore.

As far as the complete remission rate goes, I think some of her findings are intriguing. And although the numbers right now are very small, in fact, I think it would be fair to say that except for showing that three leukaphereses lower the blast count more than one leukapheresis, none of your other data is statistically significant at this point. If reducing the blast burden by a couple of fold really does increase the complete remission rate by decreasing the total tumor burden, that might be something worth knowing more about, and taking advantage of.

The other thing that, to me, is even more intriguing is the business about the growth fraction. If that really changes, if you can reduce the count by two- or threefold, and the growth fraction goes up 5 or 10 percent, making the chemotherapy more effective, that might be very intriguing, too.

The last thing I would like to comment on would be that I would be concerned about the risk of leukapheresing some of these very sick patients with high counts using either citrate or heparin in the anticoagulant, even giving them extra platelets beforehand, because a lot of these patients, if you look carefully, will have DIC as well, either overt or occult. And patients with high fibrin split products in the circulation often bleed excessively; I would be very concerned about them being at high risk, while they are getting leukapheresis, for CNS bleeding caused by the leukapheresis.

Dr. Cuttner: Well, you have mentioned several things. We very carefully study all of our patients, and DIC, though we certainly see it, has not been a problem. In fact, there are many who think that if a patient has DIC--the cause of the DIC, as we all know, is the leukemic blasts, and to the extent that you can rapidly reduce the cause, leukapheresis should help.

The other point you made was that it is true that with daunomycin and cytosine arabinoside you can certainly lower the white count very quickly in 3 days, but there are problems. One is: What do you do when your patient has a uric acid of 17? If you lower his white count in 3 days, he is certainly going to be in renal failure. So you would like to have something else to do during that period of time; and that is initially how we got into this program. But you also mentioned that you can get the counts down very quickly; your remission rate is not high in these patients. And though I certainly think the numbers are small... still, it looks very promising in that in patients who have undergone three leukaphereses, who had very high white counts, over 100,000, the remission rate was quite good.

Dr. Jacob Nusbacher: I would like to take up the cudgel for controlled studies. I think this field has suffered terribly from the fact that there are very few randomized, prospective controlled studies. I fully appreciate that many of the diseases being treated by either cytapheresis or plasma exchange are uncommon or rare, and the patients are often very sick. But wherever possible--for example, I think it is quite obvious in acute leukemia--a randomized controlled study is necessary, just on the basis of what we have heard this morning. And until we do that, I don't think we have learned anything. I am glad to see that at least the Sinai people have taken that kind of approach.

Dr. Cuttner: Thank you. I'm a big believer in that.

Dr. Taft: I think the more we have been acutely aware of this syndrome [leukostasis], I think the less we know about it. We have seen patients with relatively low blast cell concentrations in the peripheral blood who get into as much trouble as, if not more than, people who have 90 percent blasts in peripheral blood.

We have seen spleen size shrink during the course of an extended duration leukapheresis, and I think we just ought to keep in mind also that a count of 10^{12} cells is comparable to about a kilogram, plus or minus, so this is a very significant total body cell burden, recognizing there are still many cells left.

I think we need to know a lot more about the pathophysiology of the syndrome, what causes it, and I don't think it is strictly a viscosity problem or a rigidity problem of blasts, as some of the preliminary work has indicated. I think we need a lot more known on what happens--what chemotherapy does to these cells to precipitate them in the lung, or to make them in some way sticky, or to change the cell surface characteristics --whatever is going on.

Dr. Cuttner: I would certainly agree with you that there is a lot more we have to know. There is no question...that in some patients, one can lower the white count very quickly. [In] others we can't; and obviously there is a difference in the cells of those patients.

Dr. Richard Cable: I just wanted to report on one complication that I observed. We treated a lady with 570,000 white cells, with CML, with 10 percent blasts and 10 percent progranulocytes. She was having visual field losses, and we were asked to leukapherese her. We used hydroxyethyl starch and the Haemonetics Model 30. Within 50 to 100 cc of returning the first pass, she spiked a temperature of 105 and became hypotensive. We stopped the procedure. We cultured everything. She became afebrile, remained afebrile during the rest of her course. I never had any culture evidence of infection. She was treated effectively by a blood exchange transfusion done by a manual technique.

I can only attribute the fever to something that was done to her blood in the process of collecting it in the Haemonetics and then returning it to her. I don't know if anybody else has observed anything like that.

Dr. Cuttner: We have not.

Dr. Zuelzer: Do you have any closing comments, Dr. Cuttner?

Dr. Cuttner: My only closing comment is: I am a firm believer in randomized studies. I think that progress that has been made, by and large, has been done by randomized studies where the doctor can't choose what he or she thinks is the best treatment for the patient. And my own feeling is that leukapheresis does play a part in patients with acute leukemia who have very high white counts, defined as being over 100,000.

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LYMPHAPHERESIS IN THE TREATMENT OF THE LEUKEMIC PHASE OF CUTANEOUS T-CELL LYMPHOMA AND PEMPHIGUS VULGARIS*

Richard L. Edelson, M.D.
Carole L. Berger, Ph.D.
Robert B. Armstrong, M.D.
Columbia University
College of Physicians and Surgeons
New York, New York

INTRODUCTION

During the past 5 years, it has become increasingly clear that cutaneous lymphomas provide the clinician and immunologist with unique challenges and opportunities. Because the organ of primary involvement, the skin, is so accessible for observation and can be biopsied with minimal danger to the patient, and because the neoplastic cells have distinguishing morphologic and immunologic features, the mechanisms underlying the pathogenesis of these disorders may be more readily identified than those of the other lymphoproliferative processes. The regularity with which the neoplastic lymphocytes of lymphomas characterized by widespread cutaneous involvement have membrane properties generally attributed to human T-cells is quite remarkable. For this and other reasons briefly described below, we have classified this group of neoplasms as "cutaneous T-cell lymphoma" (5). This terminology has now become widely accepted and was the title of an international symposium sponsored by the National Cancer Institute in the fall of 1978.

Cutaneous T-cell lymphoma (CTCL), as a single entity, is probably far more common than had previously been recognized. At the Columbia-Presbyterian Medical Center alone, 44 new cases have been diagnosed during a 3-year period. Although this incidence may not be found in all clinical communities, CTCL certainly is not rare. Included in this broad heading of CTCL are three major subgroups: mycosis fungoides, Sézary syndrome, and atypical cutaneous lymphoma. In table 1 we have summarized a correlation of selected clinical findings and immunologic features of the neoplastic cells.

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Table 1.--Clinicoimmunologic correlations

Properties of Neoplastic Cells of Cutaneous T-Cell Lymphomas	Clinical Relevance
Affinity for skin	Widespread skin lesions; epitheliotropism
Early sparing of bone marrow	Preservation of myeloid elements
Infiltration of T-cell zones of lymphoid tissue	Effacement of normal lymphoid architecture
Depressed responses to mitogens, antigens	Primary immunosuppression
Spontaneous producers of serum macrophage inhibitory factor	Monocytopenia, abnormal monocyte chemotaxis, secondary immunosuppression
Helper T-cell function	Normal to increased serum Ig (particularly IgA and IgE), probable derivation from normal helper T-cells
Rapidly recirculating usually slow replicating	Therapeutic efficacy of leukapheresis
T-cell membrane antigens	Potential therapeutic efficacy of anti-T-cell Ig

Although CTCL has been reported to respond to a variety of chemotherapeutic and physical agents, Epstein et al. (9) were unable to demonstrate an increase in longevity of patients treated by these modalities. Encouraging responses to bleomycin (25) and azarabine (14) have been noted, however, and several prospective, well-controlled trials of chemotherapeutic agents in combinations have recently been initiated, as reported at the recent National Cancer Institute symposium. All of these clinical trials of chemotherapeutic agents are still at very early stages; at present we have no evidence of long-term remissions or cures of disseminated mycosis fungoides induced by chemotherapy. However, for disease still localized in the skin, complete remissions induced by nitrogen mustard (27) or electron beam (10) have been reported. At this juncture, the following important points can be made: First, CTCL (both leukemic and aleukemic) is generally a progressive lymphoma which appears to originate in the skin, to spread to internal structure unimpeded by local lymph nodes, and to kill the host by increased susceptibility to infection or by causing failure of vital organs. Second, once extracutaneous involvement is manifest, the disease is remarkably resistant to therapy.

LYMPHAPHERESIS

In devising new approaches to the management of patients with cutaneous T-cell lymphoma, we have attempted to exploit the distinctive features of the neoplastic cells. Five features may be particularly important: the migratory patterns of these cells, the bone marrow sparing, the slow spontaneous rate of replication of the neoplastic cells, the T-cell membrane antigens of these cells, and the presence or absence of a leukemic phase. The first of these features requires explanation. Evidence, mostly circumstantial, has accumulated to indicate that the neoplastic cells in these patients resemble normal T-cells in their propensity to recirculate through the body. As described earlier, Rappaport and Thomas have shown that once mycosis fungoides escapes the skin, the lymph nodes apparently do not serve as effective barriers against spread to other organs (22). Significantly, the following remarkable spontaneous cyclicity was observed in one of our patients: his peripheral blood lymphocyte count varied inversely with the severity of his cutaneous infiltration. As his peripheral blood lymphocyte count rapidly rose from 40,000/mm^3 to 140,000/mm^3, his skin disease significantly improved by clinical and histopathologic criteria. As his peripheral blood lymphocyte count spontaneously decreased to approximately 50,000/ mm^3, his skin disease worsened. Two such complete cycles were well documented during a 2-month period in which the patient received no chemotherapy. No evidence (increased urinary xanthines, antilymphocyte serum factors) were identified to indicate rapid cellular destruction during the phase in which the blood lymphocyte count decreased. This suggested that the neoplastic T-cells were migrating to or emigrating from the skin in waves. The factors controlling this cyclicity remain unidentified. More definitive evidence of at least rapid unidirectional flow of leukemic CTCL cells from site(s) of origin to blood to skin has been obtained from recent *in vivo* kinetic studies in three patients with the leukemic phase of CTCL (3).

As described in a preliminary report (4), extensive lymphapheresis was performed on the above leukemic patient, in an attempt to favorably alter the apparent soft tissue-blood lymphocyte equilibrium. Immediately prior to initiation of the leukapheresis, the patient's peripheral blood lymphocyte count was 216,000/mm^3 (nearly all of which were small Sézary cells), and his skin disease was clinically tumor-stage mycosis fungoides superimposed on an exfoliative erythroderma. Despite intensive chemotherapy involving several combinations of agents, the disease had been progressive before lymphapheresis was initiated. Over a 35-day period, 12 procedures were performed with complete resolution of his cutaneous lesions and a drop in his blood lymphocyte count to 21,000/mm^3. A total of 36 x 10^{11} leukocytes were removed during that period. This process was repeated 10 times during the subsequent 38 months (averaging 6 lymphaphereses per course). The patient's disease remained under good clinical control during that period despite the use of no concomitant chemotherapy or radiotherapy. Lymphapheresis of two other patients was also efficacious.

The reasons that lymphapheresis is efficacious in patients with cutaneous T-cell lymphomas, leukemic phase, are probably several. Perhaps most significantly, the neoplastic T-cells appear to migrate rapidly between the blood and soft tissue depots. Removing them in large quantity from the blood probably shifts an equilibrium and effects a relative emigration of cells from infiltrated tissue. The slow rate of replication of the abnormal cells in most patients makes it possible to remove them much more rapidly than they are being produced. Since the bone marrow reserve is largely intact, despite very high blood lymphocyte counts seen in some patients, the substantial loss of red blood cells, granulocytes and platelets which may inadvertently occur during intensive leukapheresis can be readily replaced in the peripheral blood.

Expanded Clinical Trials of Lymphapheresis

As indicated above, a pilot study of the efficacy of lymphapheresis in the treatment of the leukemic phase of CTCL was sufficiently promising to merit an expanded study of this modality. Although the factor(s) governing the apparent equilibrium between neoplastic T-cells in the tissue and blood compartments during the leukemic phase of CTCL remain to be elucidated, our pilot study had indicated that marked depletion of the peripheral blood pool of leukemic cells induced a dramatic and rapid decrease in the number of cells sequestered in the skin and peripheral lymph nodes. Our expanded study included seven patients in the leukemic phase of CTCL, and results from the treatment of six of these individuals have been reported (6). In four of the seven individuals, the disease was effectively controlled by intensive lymphapheresis in the absence of any other form of intervention. Other investigators (2,17,20) have also reported at least transient clinical responses to this modality. The first patient, whose initial course is described above, was treated with periodic leukapheresis over a 3-year period and maintained in a clinically acceptable condition with a mean blood lymphocyte count of 20,000/mm^3 and mild to moderate erythroderma. Because he had been returned to a fully functional state and because his initial skin disease had involved florid, incapacitating exfoliating erythroderma accompanied by massive disfiguring and painful cutaneous tumors, the patient and his physicians were gratified with the response to lymphapheresis. However, after a

3-year period, that individual's lymphoma entered a very aggressive lymphoblastoid phase; a rapidly progressive infiltration of all major viscera including bone marrow occurred and the patient succumbed to acute pulmonary hemorrhage.

Distinction between the good and the poor responders indicates that a low rate of spontaneous thymidine incorporation by peripheral blood mononuclear cells is a prognostic indicator (table 2): it appears that those patients with low rates of cell renewal in their peripheral blood will receive distinct benefit from intensive lymphapheresis. It is also important to emphasize that, at best, intensive lymphapheresis is an effective method of drastic tumor cell reduction in those patients with rapidly recirculating leukemic T-cells and that the natural evolution to a more aggressive neoplasm is likely to occur despite the success of lymphapheresis. Indeed, we do not yet know whether the removal of large numbers of neoplastic cells from these patients enhances such an undesirable evolution. The efficiency of lymphapheresis is also limited since below a peripheral blood lymphocyte count of 25,000/mm^3, substantial numbers of normal blood elements are also inadvertently removed. However, it is clear that lymphapheresis has a place in the management of selected patients with CTCL and that the procedure can be significantly refined. Finally, the availability from therapeutic lymphapheresis of enormous numbers of neoplastic T-cells not only facilitates a large number of studies of potential benefit to the individual patients but also provides the cellular immunologist with an invaluable source of cells for a multitude of investigations.

Table 2.--Relationship of thymidine incorporation to response to leukapheresis

Patient	Thymidine Incorporation	Response
JN	WNL	Excellent
AL	WNL	Excellent
BO	Increased	Poor
MUV	WNL	Excellent
MR	Increased	Poor
JM	Increased	Poor
RJ	WNL	Good

The encouraging results obtained with lymphapheresis in these initial studies indicated that further clinical trials of this modality were warranted. Presently, many of the clinical and immunologic sequelae of these therapeutic manipulations are largely unpredictable. Specifically, we do not yet clearly understand the effects that removal of large numbers of leukemic T-cells may have on the rate of replication and properties of residual neoplastic cells, and we do not fully appreciate the impact that removal of normal circulating lymphocytes by leukapheresis may have on the already immunosuppressed host. With respect to the administration of heterologous antithymocyte globulin, it has already been demonstrated that such antisera are capable of stimulating proliferation of some populations of CTCL cells in vitro in the absence of complement (1). Furthermore, substantial evidence supporting immunostimulation of neoplastic cells of various types has been reported (21). Our justification for pursuing these particular clinical trials in which we attempt to use distinctive cellular properties to therapeutic advantage is the simple empirical observation that studies to date suggest that these approaches may be more efficacious in the treatment of advanced CTCL than are standard chemo- or radiotherapeutic regimens.

Antithymocyte Globulin

A second feature of the neoplastic cells of CTCL which should be therapeutically exploitable is their expression of T-cell membrane antigens. Indeed, antithymocyte globulin (ATG) could be ultimately administered extracorporeally in conjunction with lymphapheresis. Such an approach would permit exposure of leukemic T-cells to high concentrations of ATG and removal of products of cell lysis before return of other elements to the patient.

In a separate pilot study (7) and then extended clinical trial (8), four patients with advanced CTCL were treated with intravenously administered horse ATG. Three of these patients had the leukemic variant while one had cutaneous and extracutaneous aleukemic CTCL. The ATG (obtained from the Upjohn Co., Kalamazoo, Michigan) had been prepared by immunization of horses with human thymocytes and has been widely used as immunosuppressive therapy in kidney transplantation programs, including that at our institution. Three of four patients clinically improved in response to ATG. One patient (EO), with widespread cutaneous plaques and tumors and massive histologically effaced peripheral lymph nodes, received ATG intermittently for 6 months. Significantly, by the end of the first month of treatment, clinical evidence of skin or lymph node disease was absent. However, minimal histologic evidence of skin infiltration persisted. No adverse effects were seen on other blood elements during this intensive treatment. Unfortunately, the ATG administration was sclerosing to her veins and ultimately had to be discontinued. Subsequently, florid disease, uncontrollable by chemotherapy, recurred, and she died of disseminated lymphoma.

A second ATG recipient, EM, had total skin erythroderma and peripheral blood T lymphoblastoid leukemia with complete bone marrow replacement. Combination chemotherapy (Price-Hill regimen) induced a dramatic decrease in peripheral blood T-cell blasts, but bone marrow remained infiltrated. Then, four infusions of ATG were administered; her marrow was repopulated by megakaryocytes and granulocyte precursors; her blood levels of platelets and neutrophils returned to normal; and her blood lymphocyte count was maintained below 20,000/mm^3. At that time, her marrow reserve was sufficient to permit subsequent management with the Price-Hill regimen. To our knowledge, she is the first patient with CTCL to survive blast crisis. *In vitro* testing revealed that the majority of normal and neoplastic (from EM) T-cells were lysed in the presence of rabbit complement up to a dilution of 1:1,000 ATG. However, human serum appeared to be a less efficient source of complement for these reactions. Significantly, normal T-cells were more sensitive to ATG at most dilutions of ATG. Cross-reactivity of normal with abnormal cells was established by the removal of most activity of ATG for normal T-cells by absorption with neoplastic T-cells.

Our findings suggest that ATG therapy may be efficacious in the management of patients with extensive CTCL. The mechanism by which ATG administration affects the neoplastic T-cells is not clear. Although *in vitro* results indicate that ATG in the presence of rabbit complement *is lytic* for both normal and neoplastic T-cells, ATG in the presence of human complement is not markedly lytic. Indeed, at dilutions equivalent to those obtainable *in vivo*, ATG is very poorly lytic for neoplastic T-cells in the presence of human complement. It therefore appears that complement-mediated lysis is not the major mechanism by which ATG induces the observed clinical responses. Alternatively, ATG coating of cells may facilitate their removal by the reticuloendothelial system or their destruction by other mononuclear cells. Identification of the mechanism(s) operative *in vivo* will require further studies.

No generalized urticaria, arthralgias, or renal function changes were noted in our patients. It is not presently clear whether individuals with advanced CTCL are capable of an immune response against the heterologous ATG. Our patients did not produce circulating antibodies to horse immunoglobulin as measured by radioimmunoassay, and no clinical symptoms of serum sickness or anaphylactoid reactions were noted. Because ATG acts at cellular sites and by mechanisms distinct from those of commonly used pharmacologic agents, its beneficial effects may be additive to those of combination chemotherapy. The important point at this juncture is that a relatively nonspecific antihuman thymocyte globulin was capable of effecting a distinct response in three patients with different clinical expressions of CTCL, without also resulting in persistent undesirable side effects. We hope to use far more specific antibodies, prepared by use of a mouse hybridoma system (13), in future trials.

LYMPHAPHERESIS PLUS PLASMAPHERESIS IN THE TREATMENT OF PEMPHIGUS VULGARIS

Pemphigus vulgaris, a disorder which was almost universally fatal prior to the use of systemic corticosteroid therapy, has been reported to respond to plasmapheresis (23). Because of the putative role of circulating antiepidermal antibodies (11,12,15,18) in the induction of the characteristic intraepidermal bullae of pemphigus vulgaris and because of the correlation between antiepidermal antibody titers in pemphigus vulgaris sera with clinical state (29), a therapeutic efficacy of plasmapheresis in this disorder would not be surprising. In the context of this symposium, it is noteworthy that several instances of pemphigus vulgaris coexisting with myasthenia gravis have been reported (16,19, 26,28).

We have recently initiated a protocol for the treatment of patients with pemphigus vulgaris by combined plasmapheresis and lymphapheresis. Since the antiepidermal antibodies react with cell surface glycoproteins (24) and since formation of antibodies against complex glycoproteins probably requires cooperation between "helper" T-cells and antibody-producing B-cells, we elected not only to remove the circulating antibody by plasmapheresis but also to attempt to deplete the lymphocytes in a pilot study.

The results of this treatment of one patient are summarized in table 3. As indicated, a remarkably rapid response was noted with complete resolution of the cutaneous disease accompanied by a drop from 1:1,280 to 0 of circulating antiepidermal antibody. Since that patient had suffered from intractable disease for more than 3 years under observation of one of us, we consider it quite unlikely that the response to therapy was simply fortuitous. Serious disease returned several weeks following combined plasma- and lymphapheresis, indicating a requirement for continued therapy. The remarkable drop in titer can not be explained on the basis of simple antibody removal by two treatments and may reflect a combination of dramatically reduced production following acute lymphocyte depletion as well as sequestration of other antibody in the epidermis. On the basis of these promising results, we intend to substantially expand this study to include both other patients with pemphigus vulgaris and a comparison between combined plasmapheresis and lymphapheresis and plasmapheresis by itself.

Table 3.--Results of combined treatment

	Titer IC IgG	Clinical Status
Pretreatment	1:1,280	4+ involved
Post-treatment	1:80	Pruritus resolved Lesions persist
2 weeks post-treatment	not detectable	Skin clear
3 months post-treatment	1:160	3+ involved

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DISCUSSION

Dr. Zuelzer: This paper is now open for comments and questions.

Dr. Ries: I found that a very provocative presentation. Let me ask you a couple of things on your definitions. Are you calling patients with erythroderma that look like mycosis fungoides but don't have circulating abnormal cells, but have a typical cutaneous infiltration, your cutaneous T-cell lymphomas, but not Sézary's syndrome? Is that what those other 27 are? Or however many there are?

Dr. Edelson: One of the major reasons for introducing the term "cutaneous T-cell lymphoma" to replace "Sézary syndrome," "mycosis fungoides," and "cutaneous reticulosis" is that cellular immunology has taught us that they are all part of the same spectrum of disease. Cutaneous T-cell lymphoma specifically refers to malignant monoclonal expansions of helper T-cells which preferentially infiltrate the skin but ultimately disseminate to viscera. Mycosis fungoides is one major aleukemic variant of this disorder, while the Sézary syndrome is a leukemic phase of the process. However, most of the patients with cutaneous T-cell lymphoma do not present with clinical or histopathologic features characteristic of either mycosis fungoides or the Sézary syndrome, and have previously been artificially splintered from the whole group. Now that we appreciate that cutaneous T-cell lymphoma is a single type of neoplasm with varying expressions, it has become clear that the incidence of the process approximates that of Hodgkin's disease.

Dr. Ries: I think in the symposium that they had at the Mayo Clinic here several years ago, which was published, they had a disorder also called "pre-Sézary syndrome," which may be what you are describing. I don't mean to make an issue about this, except I am a clinician, and I see a lot of patients for the dermatology service that have erythroderma, this kind of a skin infiltrate, pretty much a normal peripheral blood picture, and the lymph nodes may or may not be involved. And these patients are miserable, and need some kind of treatment which, up until now, we haven't had very much treatment for, or I haven't been very impressed by the treatment we have had for it.

Now, unfortunately, these patients aren't in the leukemic phase; so your approach, then, of lymphapheresis doesn't seem that it would be useful in that situation. Do you have any ideas, or do you have any experience, in how to get the cells out of the skin into peripheral blood so that they become available for mechanical separation?

Dr. Edelson: Typically, patients with cutaneous T-cell lymphoma have localized or generalized skin disease for several years prior to histopathologic diagnosis. This most likely reflects the limitation of our diagnostic techniques, rather than the existence of a true pre-Sézary syndrome. As our ability to diagnose the Sézary syndrome has become progressively more refined, the number of patients classifiable as "pre-Sézary syndrome" has become smaller.

A large number of patients with idiopathic exfoliating erythroderma have an incapacitating disorder which cannot be clearly classified as Sézary syndrome, even though they generally have a few atypical circulating lymphocytes. Your assertion that those patients do not respond to lymphapheresis is in agreement with our experience, but their disease can often be markedly palliated by a combination of prednisone and imuran. The group at the Mayo Clinic has obtained encouraging results in this type of patient with the use of low-dose chlorambucil, although significant response may not occur for several months.

Dr. Ries: Our experience has been that some do and some don't, or often they will do well for a while, and then later on they will have more refractory disease. We haven't seen a high rate of conversion to systemic aggressive T-cell lymphoma. Are your conclusions based on many of the patients of your 34 patients, or is that only a few that you have seen so far that have actually progressed, and over how long a period of time, on the average?

Dr. Edelson: We have established a special logistical situation which explains the relatively large number of patients with cutaneous T-cell lymphoma that we see. The residents, full-time attendings, and practitioners associated with our medical center are particularly aware of this type of lymphoma and are quick to identify it. Virtually all of the patients with cutaneous lymphoma diagnosed by members of our medical community are referred immediately to the clinical facility of the Columbia-Presbyterian Cancer Research Center. By centralizing care of patients with cutaneous T-cell lymphoma of all types of presentation, we have been able to determine that the frequency of this disease is at least equal in our medical community to that of Hodgkin's disease.

Dr. Frank Jones: In your one patient that you saw that had such a dramatic reduction of tumor load, the T-cell recirculating guy that was alive for 3 years--this is kind of a theoretical question, but could it be that the first tumor was one that responded very well to your treatment, but the second kind, the relapse, may have been a different kind of tumor that would not respond to therapy--which would skew your thinking as to the therapy protocol?

And the second question is: If, in this kind of tumor that you were looking at, there was indeed a recirculating T-cell, what would you feel would be the response to...chronic thoracic duct drainage?

Dr. Edelson: By longitudinally following patients with cutaneous T-cell lymphoma, we have been able to determine a natural evolution of the process to one of progressively increasing malignancy. Therefore, we would agree that it is quite likely that the ultimate failure of treatment in that patient after 3 years of substantial success was a direct reflection of the evolution of subclones of more malignant T-cells with decreased tendency to rapidly recirculate. Indeed, at the time of autopsy, the peripheral blood lymphocyte count of that patient was less than 20,000 per cubic millimeter, while virtually all visceral structures were markedly infiltrated by malignant T-cells. It seems that the equilibrium shifted in favor of the infiltrative component of the disease.

In answer to your second question, thoracic duct drainage has theoretical advantages over lymphapheresis. However, the morbidity is much greater and canulation cannot be repeatedly performed.

Dr. Goldfinger: A comment in relation to what you just said: We have considerable experience in treating chronic lymphocytic leukemia on a long-term basis. One, of course, was your patient, J. N., with T-cell lymphoma, and 9--or 11 others, rather--with B-cell disease. And as you know, long-term therapy using leukapheresis for chronic granulocytic leukemia has been unsuccessful for two reasons. First, the disease converts to an accelerated phase of blast crisis, and second, because the production of abnormal granulocytes in that disease is so rapid that you can't keep ahead of the disease.

We have been led to believe that in the lymphoproliferative disorders, we don't face those problems: number one, that the disease does not convert to a more aggressive form; and second, that production of abnormal cells is rather slow.

Our experience, and lack of success, with the treatment of these patients--by the way, we are having trouble publishing this material, although we did present it at the American Association of Blood Banks meetings in November. I think it is important...that the journals allow for publication of negative studies. Negative studies have always been hard to get published; but in this field, where we see this blooming of positive results, and yet here at the meeting, we hear about a couple of cases of TTP, for example, that did not respond to therapy, it is important that these be published.

At any rate, we could not get anywhere with our treatment; and in J. N.'s case, we saw a transformation to probably a more aggressive disease. I think we saw that with a couple of the B-cell cases, as well.

In addition, we could not get ahead of the disease, even though we removed huge numbers of lymphocytes. Now, either the production rate of these cells is much greater than we have ever thought, or removal of the cells causes increased production, maybe by removal of negative feedback. Or perhaps, as you have indicated, we are underestimating the total tumor burden, as you have shown, in the skin. It is very large. Maybe the tumor burden in our cases was so huge that even though we remove kilograms of material, we could see no objective reduction, for example, in spleen size or liver size or node size.

Dr. Edelson: We feel that those comments have substantial merit. We have recently completed a kinetic study in which we injected tritiated thymidine into the circulation of patients with cutaneous T-cell lymphoma and then determined rates of cell renewal by sampling of skin or blood. The autoradiographs indicated that with advanced disease an extraordinarily rapid rate of proliferation occurs in sites other than the skin or blood. The most likely sites of this proliferation are lymph node and spleen. Lymphapheresis is successful as cytoreductive therapy only when one can remove cells from the peripheral blood more rapidly than they are being produced.

Dr. Khan: First I would like to do an apheresis of a confusion I created...I wasn't against controlled studies. What I meant was...we should be careful what kind of controls we have. I think every study should be controlled if it possibly can be, and we should have randomized trials.

Now, my question is: The project that you outlined, where you would like to treat these lymphocytes extracorporeally with the anti-T-cell antiserum--I can't understand how we could achieve some benefit from it, because these cells already are out. You could easily remove them; and why would you like to treat them with antiserum and reinject them...you are going to wash any excess antiserum anyhow, so that antiserum is not available to treat, or to affect, any other cells in circulation.

Dr. Edelson: We are not as efficient as we would like to be in removing the lymphocytes as they pass through the machine. Our calculations indicate that we rarely obtain more than 50 percent of the cells that circulate extracorporeally. If one could add the anti-T-cell antibodies to the apparatus, the efficiency of T-cell removal and/or destruction might be markedly enhanced.

Dr. Richard Branda: Whether excessive helper T-cell function or lack of suppressor T-cell function, it seems that changes in lymphocyte populations are important in autoimmune disease. I don't know that anyone has studied systematically the effect of plasma exchange on T-cells, except one paper in the German literature that suggests that there was no change. I wonder if, in your patient with pemphigus, or the other patients, you have looked systematically at T-cell or other lymphocyte populations--at whether there is a change.

Dr. Edelson: We agree that careful studies of this type would be quite valuable. The groups who remove plasma tend to study plasma factors, and the groups who remove lymphocytes tend to study lymphocyte function. It would be quite important for the studies of both types of groups to be more inclusive.

Dr. Zuelzer: Thank you, Dr. Edelson....

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CYTAPHERESIS FOR PROCUREMENT OF EOSINOPHILS AND FOR THE TREATMENT OF DISEASES ASSOCIATED WITH EOSINOPHILIA*

Gerald J. Gleich, M.D.
Alvaro A. Pineda, M.D.
Graham O. Solley, M.D.
Howard F. Taswell, M.D.
Mayo Medical School
Mayo Clinic and Foundation
Rochester, Minnesota

INTRODUCTION

In 1879, Paul Ehrlich described a peripheral blood cell which stained brilliantly with acidic dyes, and he termed this cell "acidophil or eosinophil" (1). Now, 100 years later, the functions of the eosinophil remain shrouded in mystery (2,3). Very recent evidence points to a beneficial role for the cell in immunity to parasites (4-9).

For several years we have investigated the proteins of the eosinophil. Initially our work concentrated on guinea pig eosinophils and resulted in the identification of a major basic protein from the eosinophil granule (10,11). Subsequently we investigated Charcot-Leyden crystals, a material unique to human pathology and formed by the eosinophil (2), and compared the Charcot-Leyden crystal protein to the human major basic protein (12). To obtain the large number of eosinophils needed for investigation of their cytoplasmic proteins and for determination of the effect of removal of eosinophils on disease, we used continuous-flow and semicontinuous-flow cell centrifugation systems. A report of certain of this information has already been published (13).

MATERIALS AND METHODS

We used two different devices for obtaining eosinophils, the NCI-IBM continuous-flow cell separator and the Haemonetics semicontinuous-flow cell processor. With the NCI-IBM cell separator, Model 2990, blood was removed from either antecubital vein and was anticoagulated with acid citrate dextrose-NIH formula A at a 1:4 ratio. The blood was pumped at a rate of 50 ml/min through the centrifuge bowl and returned by way of

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the antecubital vein of the opposite arm. With the Haemonetics cell processor, Model 10 or 30, whole blood was removed from the donor, and after addition of acid citrate dextrose or citrate anticoagulant the blood was pumped into a transparent disposable centrifuge bowl rotating at a speed of 4,800 rpm. In all of the experiments employing the Haemonetics system the anticoagulant used consisted of sodium citrate 2 percent added to 6 percent hydroxyethyl starch (HES). A detailed description of both the continuous- and semicontinuous-flow systems has already been presented (13).

The treatment protocols were approved by the institutional human studies committee, and in all cases informed consent was obtained from patients prior to cytapheresis.

RESULTS

The results from 19 cytaphereses in 11 patients are presented in table 1. These results warrant comment in two regards. First, concerning the diseases associated with eosinophilia, patients number 1 through number 7 have already been described (13). Since that report, patient 2 (number 10 in reference 13) has developed a lymphoma, patient 4 (number 6 in reference 13) has been effectively treated with prednisone on an every-other-day regimen, patient 5 (number 9 in reference 13) has been treated intermittently with prednisone and hydroxyurea and is still alive 4-1/2 years after diagnosis, and patient 6 (number 11 in reference 13) has required treatment with corticosteroids and indomethacin, up to 120 mg a day, and his symptoms have been well controlled. In all of these patients the relationship between the eosinophilia and the patient's symptomatology was not clear except, perhaps, in patients 1 and 6. In these latter patients, the degree of eosinophilia appeared to be directly related to the severity of the patient's symptoms; the symptoms were clearly worse when the levels of eosinophils were highest. Patients 9 and 10 had eosinophilia associated with hyperplasia of many elements of the bone marrow, and patients 8 and 11 had eosinophilia without a clear-cut relationship to their symptoms. Second, it appears that the use of HES considerably improves the recovery of eosinophils. For example, in patients 1, 2, 3, 5, and 6 the efficiencies of eosinophil recovery were quite low, whereas in patient 7, where HES was used in all cytaphereses, the recoveries were considerably higher. In table 2 the calculated efficiencies of recovery of eosinophils are presented. Clearly, the addition of HES improves the recovery of eosinophils as judged by the comparison of cytaphereses performed in patients on the continuous-flow device with and without HES (p=0.01, Rank sum test). Similarly, comparison of the eosinophil recoveries between the continuous-flow apparatus not using HES and the semicontinuous-flow with HES revealed a difference (p=0.02, Rank sum test). In contrast no difference was found between the two devices when HES was used in the anticoagulant

Table 1.--Results of eosinapheresis

Patient Number	Diagnosis	Leukocyte Cells per µl	Leukocyte Eosinophils Percent	Blood Processed ml	Eosinophils Recovered x10^9	Efficiency of Removal[a] Percent	Device[b]	HES[c] Used
1	Eosinophilic pneumonitis	19,750	60	4,877	8.88	15.4	CF	No
2	Dermatitis and lymphoma	30,600	67	3,500	1.83	2.6	CF	No
3	Vasculitis	12,900	25	10,800	0.389	1.1	CF	No
4	Angioedema and leg nodules	10,100	68	9,285	6.14	9.6	CF	Yes
5	Hypereosinophilic syndrome	26,400	53	10,000	4.20	3.0	CF	No
		15,000	61.5	10,000	4.81	5.2	CF	No
		14,500	63	10,000	2.20	2.4	CF	No
6	Angioedema	34,700	61	10,000	24.8	11.7	CF	No
		37,800	63	10,000	45.5	19.3	CF	No
7	Lymphoma and fibrotic nodules	13,000	57.5	2,730	11.2	54.0	CF	Yes
		10,300	64	9,535	17.6	28.0	CF	Yes
		10,000	57.5	10,000	21.6	37.6	CF	Yes
		12,000	67	7,639	21.8	35.5	CF	Yes
8	? Hypereosinophilic syndrome (oral and genital ulceration)	11,400	46	2,814	32.2	218.0	SCF	Yes
		9,000	67.5	4,581	26.8	96.3	SCF	Yes
9	? Myeloproliferative syndrome	5,700	59.5	4,464	1.78	11.7	SCF	Yes
10	Myeloproliferative syndrome with basophilia	26,400	13.5	5,966	11.0	51.8	SCF	Yes
11	Hypereosinophilic syndrome with wasting	21,100	80.5	4,613	12.1	15.4	SCF	Yes
		16,600	80	3,922	7.49	14.4	SCF	Yes

[a] Efficiency is calculated by dividing the number of eosinophils passed through the system by the number of eosinophils recovered x 100. Blood counts before and at the end of the procedure revealed that the level of eosinophils was essentially the same.

[b] CF = Continuous flow; SCF = Semicontinuous flow

[c] HES = Hydroxyethyl starch

solution. Thus the inclusion of HES is an important determinant of the efficiency of eosinophil recovery by cytapheresis. Comparison of results in individual patients indicates that, in addition to the effect of HES, the characteristics of a patient's own eosinophils are important in determining whether or not efficient recovery was achieved. For example, patient 8 had 218.0 and 96.1 percent of his eosinophils recovered on two cytaphereses utilizing the semicontinuous-flow centrifugation and HES, while patients 9, 10, and 11 had considerably smaller percentages of their eosinophils recovered under identical conditions of cytapheresis. Finally, the 218 percent recovery in the first cytapheresis in patient 8 suggests that eosinophils were mobilized from extravascular sites, presumably bone marrow or tissue stores.

Table 2.--Comparison of eosinapheresis procedures

Cytaphereses Number	Number of Patients	Device	HES	Recovery of Eosinophils Efficiency % ($\bar{X} \pm$SEM)
8	5	CF	No	7.6±2.5
5	2	CF	Yes	32.9±7.2
6	4	SCF	Yes	67.9±32.9

DISCUSSION

Cytapheresis is an effective means of depleting blood cells in a variety of conditions (14). Application of this procedure to the treatment of patients with eosinophilia has proven disappointing. However, this pessimistic view should be tempered by the recognition that many of the patients shown in table 1 had clinical situations in which one would not expect dramatic results. For example, patient 7 presented with hard nodules over the scalp and sternum which, though infiltrated with eosinophils, were composed primarily of dense fibrous tissue. In this patient, even if one had been able to successfully deplete tissue eosinophils, one would not have expected to alter dramatically the fibrotic lesions. Also, inspection of table 1 indicates that in most cases the efficiencies of eosinophil recovery were so low that the total numbers of these cells in the peripheral blood were not altered. Only in patients 7, 8, and 10 were truly significant proportions of the total number of circulating eosinophils removed, and only in patient 8 was there an indication that eosinophils were mobilized from extravascular sites. Therefore, even though these results appear discouraging, one must keep in mind the possibility that more intensive cytapheresis utilizing HES in the anticoagulant might permit more efficient recovery of cells and thus amelioration of the underlying clinical problem.

On the other hand, the eosinophils recovered by cytapheresis have been most useful for the investigation of eosinophil proteins. For example, we have been able to purify a major basic protein from the human eosinophil granule (12) which shares properties in common with that derived from the eosinophil granules of the guinea pig (10,11) and the rat (15). In addition, we have been able to produce Charcot-Leyden crystals from human eosinophils and have shown that Charcot-Leyden crystal is composed of a protein with a molecular weight of 13,000 containing 1.2 percent carbohydrates (12). Comparison of the properties of the Charcot-Leyden crystal protein and the major basic protein from human eosinophils reveals that these differ in their molecular weights, carbohydrate compositions, and amino acid analyses. Thus, on the basis of their physicochemical properties, these are distinct materials because one cannot be derived from the other. We have recently shown that the eosinophil major basic protein directly damages schistosomula of Schistosoma mansoni (16) and may well play a major role in mediating the presumed beneficial role of the eosinophil in resistance to parasites. Previously the major basic protein had not been shown to have any biologic function apart from its capacity to neutralize heparin and to activate papain (11). Yet this protein accounts for over 50 percent of the eosinophil granule's protein and about 25 percent of the total cellular protein, and presumably has an important part to play in the function of the eosinophil. Finally, further investigations of human eosinophil proteins are dependent on the availability of large numbers of these cells, and these numbers can be obtained only through the use of either continuous-flow or semicontinuous-flow cytapheresis using HES.

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DISCUSSION

Dr. Zuelzer: This most fascinating paper is now open for discussion. I came away, if I may say this first, with the impression that maybe you shouldn't even try to remove the eosinophils, because you have convinced us that they serve a very useful purpose.

Dr. Gleich: The work of several investigators has shown that eosinophils have the capacity to kill larval forms of certain parasites, including schistosomules of Schistosoma mansoni and newborn larvae of Trichinella spiralis. Therefore, removal of eosinophils might be undesirable in that it could diminish resistance to parasites. On the other hand, our data indicate that appreciable concentrations of the major basic protein can be found in body fluids. Because of the potential toxicity of the major basic protein we have wondered whether the eosinophils through the major basic protein might damage tissues in disease.

Dr. Goldfinger: Schistosomiasis, of course, is one of the great plagues of the world, and an important disease; but I have learned from this lecture to give it even greater respect. Any organism that is able to lie in continuous copulation certainly deserves our respect. I do have a question, however. I was called by a friend in Los Angeles. He had a patient with hypereosinophilic syndrome with a count of about 200,000. He tried to apherese him, and noticed that the buffy coat that he collected had a lower eosinophil percentage than the patient's blood; and I told him the only people I knew who were doing eosinapheresis was the group at Mayo, and I told him I would ask you. I think you have answered the question.

We always assume that white cells are in the buffy coat, but it may be that in this case, eosinophils have a density more like red cells and are indeed not in the buffy coat. And, of course, you demonstrated that when you add hydroxyethyl starch, which he did not add, by changing the characteristics of the red cells, suddenly we can separate the two.

Dr. Gleich: It also may be, and I would appreciate Dr. Taswell's comment on this, it may be that we are dealing, in certain diseases, with eosinophils in different stages of maturity and possibly with different surface properties or different densities.

Dr. Howard Taswell: Two points: One, your comment relates to a comment made earlier--I think Dr. Gilcher made it--about the difference in the behavior of different types of granulocytes and blasts, and it's exactly the same comment, essentially for eosinophils in different stages of maturity. The other point, relative to to the need for both negative and positive studies: To the best of my knowledge, there had only been one case report in JAMA, a few years ago, reviewing a single patient treated in this manner with a very optimistic report of the usefulness of eosinapheresis. Our results certainly are quite negative and very much in contrast to that one case reported in JAMA.

Dr. Gleich: I should comment that the patient in the case report in JAMA may have had an eosinophilic vasculitis, and this may have been in the downswing of the disease activity. Therefore, doing a cytapheresis in a patient who was spontaneously recovering would lead one to conclude that cytapheresis was a lovely therapy. Of course, I don't know that, but from my reading of the patient's history, I believe it is a not unreasonable surmise.

Dr. Khan: I wasn't clear if the effect of the eosinophilic protein was mediated through the IgE,...or IgG, or whether the antibody had to be bound to the schistosome before the protein is toxic. If this is true, then have you tested, or are you planning to test, the effect of such a protein on any other antibody-mediated lysis...? And secondly, did you culture, say, normal cells of any sort and add protein in any given concentration to see if it is toxic, at any level at all?

Dr. Gleich: In answer to your first question, one does not need the IgG antibody. We appear to be circumventing the normal process by which the eosinophil attacks the parasite by adding major basic protein directly to the schistosomule. Normally, the recognition of the parasite by the eosinophil is mediated through the IgG Fc receptors, which binds the eosinophil to the parasite.

In answer to your second question, we have found that major basic protein is toxic to a variety of cells and we have suggested that eosinophils may damage tissues in disease and that the major basic protein may be a mediator of this damage.

Dr. Khan: Can I ask one more question? The reason I was bringing it up: many allergists will swear that the patients with allergy have a very low incidence of malignant diseases.

Dr. Gleich: Of malignant diseases?

Dr. Khan: Of malignant diseases. And I have heard many allergists say that. I don't know if there is any statistical analysis or controlled study on that. But if it is so, most patients with allergies would have very high eosinophil counts, and I wonder if this protein might have something to do with it. That is why I was asking, if this is direct cytotoxic effect, or any other effect, on the transformed cell....

Dr. Gleich: The function of the eosinophil, in diseases including lymphomatous diseases, gastrointestinal diseases, cutaneous diseases, allergic diseases, remains obscure at the present time.

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PLATELETAPHERESIS IN THE TREATMENT OF THROMBOCYTHEMIA

Edwin G. Taft, M.D.
Albany Medical Center Hospital
Albany Medical College
Albany, New York

INTRODUCTION

Thrombocythemia in association with myeloproliferative disorders frequently presents as a hemorrhagic or thrombotic symptom complex, requiring urgent cytotoxic and/or radioisotopic therapy. Optimum responses to marrow-suppressive therapy usually require a minimum of 2 to 3 weeks, during which time the patient's symptoms may worsen, resulting in increased morbidity and, occasionally, mortality. Should the initial therapy be insufficient, the patient may remain at increased risk of hemorrhagic and/or thrombotic complications for a longer period. The addition of antiplatelet medications may contribute to symptomatic relief in some patients with thrombotic symptoms, but it appears to be most effective in patients with platelet counts less than 1,000,000/μl. Rapid reduction of elevated platelet counts to well below 1,000,000/μl appears to be the most satisfactory treatment of symptomatic thrombocythemia.

Colman first described the value of plateletapheresis as initial therapy in patients with symptomatic thrombocythemia (2). Decrement in platelet counts from 980,000 to 1,615,000/μl before pheresis to 560,000 to 740,000/μl after apheresis were achieved by manual plateletapheresis in three patients. Symptomatic improvement (cessation of bleeding) was noted in all three patients. The importance of urgent control with cytotoxic drugs was stressed, and full doses of nitrogen mustard and/or busulfan were used.

Table 1 summarizes previous reports in the literature of experiences using plateletapheresis for urgent control of thrombocythemia. In only a relatively few reports are there enough data to abstract information sufficient to determine the efficacy of the procedure and evaluate the role of plateletapheresis in the management of thrombocythemia. With the large number of centers known to be doing therapeutic apheresis procedures, it is probable that unpublished accounts outnumber reported cases.

Table 1.--Summary of therapeutic plateletapheresis reports

Reference	Patient Number (age, sex)	Diagnosis[a]	Platelet Count (/µl)		Number of Aphereses	Method[b]	Medical Therapy	Day Postapheresis #1 of First Platelet Nadir
			Preapheresis	Postapheresis				
2	1 (66, M)	PV	980,000	560,000	1	Man	HN_2; busulfan	14
	2 (45, F)[c]	CML	1,505,000	590,000	1	Man	busulfan	31
	3 (63, F)[c]	UMPD	1,615,000	740,000	1	Man	HN_2; busulfan	9
7	1 (NS)[d]	1° Thr.	>1,500,000	"normal"	5	CFC	NS	NS
	2 (NS)	1° Thr.						
5	1 (55, M)[c]	PV	2,700,000- 4,250,000	1,800,000- 2,694,000	3	IFC	hydroxyurea	20
	1 (55, M)[c]	PV	3,510,000- 4,063,000	1,190,000- 2,300,000	3	IFC	hydroxyurea	NS
8	16 (47, M)	AMM	1,320,000	770,000	2	IFC	HN_2	10
	17 (87, M)	?1° Thr.	970,000	377,000	2	IFC	NS	NS
9	(See first five patients in table 4.)							
1	1 (23, F)	CML	1,790,000	492,000	5	IFC	——	—
3	1 (NS)	(NS)	1,400,000	600,000	3	IFC	busulfan	NS
10	1 (75, F)	1° Thr.	2,300,000- 3,500,000	835,000- 1,400,000	3	IFC	HN_2; busulfan	61
4	4 (63, M)[c]	PV > AML	2,468,000	(NS)	(NS)	(NS)	cytosine; 6TG	NS
6	6 (80, F)	(NS)	1,180,000	"normal"	?1	(NS)	HN_2; busulfan	NS

[a] PV = polycythemia vera; CML = chronic myelogenous leukemia; UMPD = undifferentiated myeloproliferative disease; 1° Thr. = primary thrombocythemia; AMM = agnogenic myeloid metaplasia; AML = acute myelogenous leukemia.

[b] Man = manual; CFC = continuous-flow centrifuge; IFC = intermittent-flow centrifuge.

[c] Patient splenectomized prior to first plateletapheresis.

[d] NS = information is not stated in the report.

While centrifuges can accomplish significant reductions in elevated platelet counts in a few hours, there remain problems. Future technical developments and additional sophistication in patient diagnosis and selection may offer improvement in the present management of thrombocythemia.

PATIENT SELECTION

Eighteen patients aged 22 to 88 with initial platelet counts greater than 1,000,000/µl, 15 with symptoms attributed to thrombocythemia, were treated with 1 to 6 plateletaphereses. These patients represent approximately 10 percent of all patients with platelet counts in excess of 1,000,000/µl in our hospital, plus others selectively referred from area hospitals specifically for plateletapheresis.

The diagnoses of these patients are shown in table 2. Five patients had undergone splenectomy 1 week to 4 years prior to the first plateletapheresis. Two of four patients believed to have had primary thrombocythemia have subsequently (18 to 30 months after the first plateletapheresis) shown evolution toward polycythemia vera. The most recent patient was initially thought to have had primary thrombocythemia, but has since been shown to have a reactive thrombocytosis.

The magnitude of elevation of platelet counts is shown in figure 1. Only 2 of the 18 patients had initial platelet counts greater than 3,000,000/µl ("severe" thrombocythemia), while the remainder had initial platelet counts from 1,000,000 to 3,000,000/µl ("moderate" thrombocythemia).

The indications for plateletapheresis or symptoms attributed to thrombocythemia in the 18 patients are listed in table 3. Unless there was some urgent reason (such as imminent extended travel of one patient listed under "pre-emptive"), asymptomatic patients were not treated by plateletapheresis except preoperatively.

METHODS

Vascular access was achieved primarily by peripheral venipuncture. Where peripheral veins were inadequate for sufficient flow, percutaneous femora venous catheters (Seldinger technic/Shaldon catheters) were used. In two patients, A-V shunts were utilized, and in one patient an A-V fistula previously created for hemodialysis was used.

Table 2.--Diagnoses of patients treated by plateletapheresis

Number of Patients	Diagnosis	Comments
6	Polycythemia vera	2 postsplenectomy
4	Chronic myelogenous leukemia	1 postsplenectomy; 1 postrenal transplant
4	1° Thrombocythemia[a]	
3	Other myeloproliferative[b]	2 postsplenectomy
1	Reactive thrombocytosis	Inflammatory bowel disease with pericarditis, pleural effusions

[a] Two of these patients have begun to show evolution toward other myeloproliferative disorders (polycythemia vera) 18 to 30 months after initial plateletapheresis.

[b] Includes myelofibrosis and undifferentiated myeloproliferative disease.

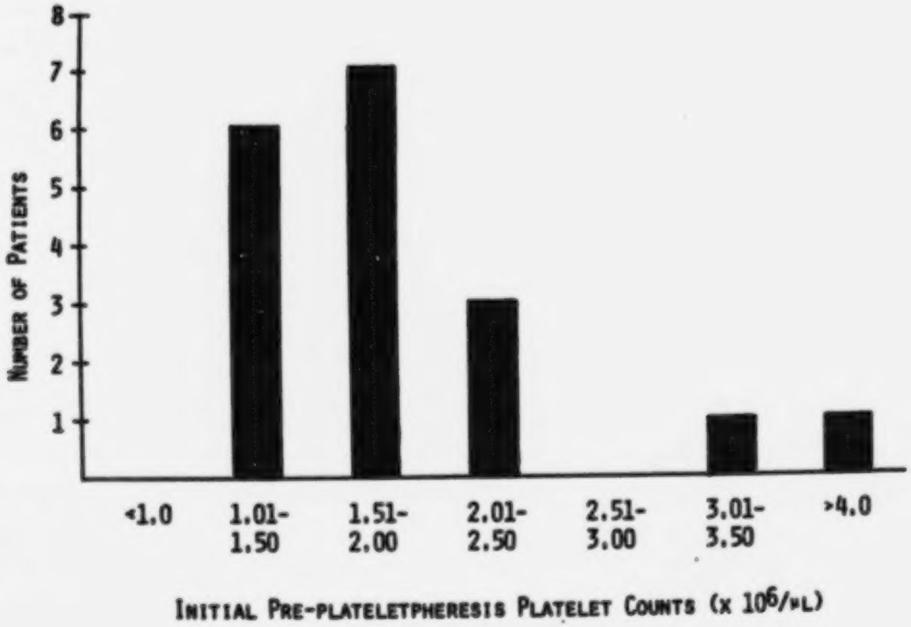
Table 3.--Symptoms and/or indications for plateletapheresis

Symptoms/Indications	Number of Patients
"Thrombotic"	
Localizing neurologic signs (TIA, CVA)	3
"Soft" neurologic signs[a]	3
"Hand-foot" syndrome[b]	2
Pulmonary emboli	2
Angina (severe, prolonged)	1
Pericardial syndrome	2
"Hemorrhagic"	
Gastrointestinal bleeding	3
Extensive subcutaneous bleeding	1
Preoperative	
Acute abdomen (perforated ulcer)	1
Subarachnoid hemorrhage	1
Elective (drainage of lymphocele)	1
Pre-emptive	
Prior to extended travel	1

[a] Describes decreased state of consciousness without localizing neurologic signs, usually in elderly patients, responding to plateletapheresis.

[b] Refers to painful, plethoric extremities (erythromelalgia).

Figure 1.-- The distribution of degree of thrombocythemia in 18 patients is shown. Thrombocythemia less than 1,000,000/µl is considered "mild"; between 1,000,000 and 3,000,000/µl, "moderate"; and greater than 3,000,000/µl, "severe."



A continuous-flow centrifuge (CFC; Aminco Celltrifuge) was used for all except the first two patients, for whom an intermittent-flow centrifuge (IFC; Haemonetics model 10) was used. For continuous-flow centrifugation, a bowl speed of 1,500 rpm resulted in clear platelet separation, and platelets were removed through the WBC ports.

RESULTS

The patients and details of their plateletaphereses are listed in table 4. Concomitant and subsequent medical therapy, and the rapidity of the first platelet count nadir achieved by médical treatment are also listed.

A summary of several parameters of plateletapheresis procedures is shown in table 5. A mean platelet decrement of 1,108,000/µl was achieved, requiring the passage of more than 9 1 of blood (most frequently equivalent to two or more blood volumes of the patients being treated).

Table 4.--Summary of patients treated by plateletapheresis at Albany Medical Center Hospital

Patient Number	Age, Sex	Diagnosis[a]	Platelet Count (/µl)		Number of Aphereses	Method[c]	Medical Therapy[d]	Day Post-apheresis #1 of First Platelet Nadir
			Preapheresis	Postapheresis				
1	(43, F)[b]	PV	1,504,000- 4,200,000	1,200,000- 1,960,000	6	IFC	^{32}P; TEM; hydroxyurea	49
2	(54, M)[b]	CML	1,400,000- 1,705,000	850,000- 900,000	2	IFC	^{32}P; hydroxyurea	21
3	(64, F)[b]	PV MF	1,182,000- 1,275,000	470,000- 475,000	3	CFC	^{32}P; melphalan	56
4	(56, M)	PV	990,000- 2,165,000	410,000- 527,000	3	CFC	^{32}P; hydroxyurea	28
5	(47, F)	1 °Thr.	1,026,000- 1,250,000	275,000- 356,000	3	CFC	melphalan	32
6	(64, M)	CML	1,956,000- 2,200,000	400,000- 860,000	3	CFC	^{32}P; hydroxyurea	38
7	(21, M)	CML	1,055,000- 1,665,000	468,000- 495,000	2	CFC	hydroxyurea; azathioprine[e]	11
8	(72, M)	UMPD	1,360,000- 1,900,000	457,000- 740,000	3	CFC	melphalan; hydroxyurea	41
9	(62, M)	PV	1,026,000	537,000	1	CFC	^{32}P; hydroxyurea	40
10	(60, F)	1° Thr.	1,250,000	450,000	1	CFC	hydroxyurea	15
11	(57, M)[b]	MF	1,875,000	920,000	1	CFC	melphalan	29
12	(82, F)	CML	1,400,000- 1,990,000	315,000- 1,060,000	4	CFC	busulfan; ^{32}P; hydroxyurea	68
13	(63, M)[b]	UMPD	1,568,000-	460,000-	1	CFC	^{32}P	29
14	(88, M)	1° Thr.	1,665,000- 3,700,000	320,000- 745,000	2	CFC	^{32}P; melphalan	52
15	(73, M)	1° Thr.	2,400,000	770,000	1	CFC	hydroxyurea	15
16	(80, F)	PV	1,760,000	500,000	1	CFC	hydroxyurea	42
17	(71, F)	PV	715,000- 1,680,000	289,000- 660,000	2	CFC	^{32}P	—
18	(26, M)	Reactive Thrombocytosis	1,170,000- 1,200,000	490,000- 495,000	2	CFC	————	—

[a] PV = polycythemia vera; CML = chronic myelogenous leukemia; MF = myelofibrosis; 1° Thr. = primary thrombocythemia; UMPD = undifferentiated myeloproliferative disease.

[b] Patient splenectomized prior to first plateletapheresis.

[c] IFC = intermittent-flow centrifuge; CFC = continuous-flow centrifuge.

[d] ^{32}P = radioactive phosphorus^{32}; TEM = triethylenemelamine.

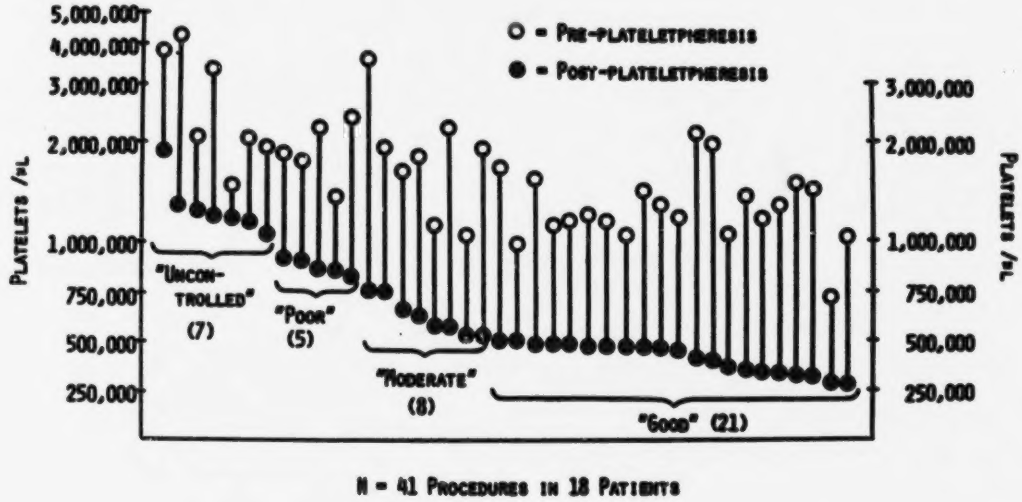
[e] azathioprine given for immunosuppression postrenal transplantation.

Table 5.--Summary of plateletapheresis procedures

	Mean	Range
Preapheresis platelet count (/μl)	1,773,000	715,000 - 4,200,000
Postapheresis platelet count (/μl)	666,000	275,000 - 1,960,000
Decrement in platelets (/μl)	1,108,000	279,000 - 2,975,000
Liters of blood processed	9.45	2.97 - 17.1
Blood volumes passed	2.31	0.59 - 3.91
Duration in hours	3:47	2:20 - 5:00
Yield (x 10^{12} platelets)	4.1	1.6 - 9.8
Yield (x 10^{12} platelets/M^2)	2.4	1.0 - 5.3
Yield (x 10^{12} platelets/liter blood processed)	0.46	0.13 - 0.87

The adequacy of reduction achieved by each plateletapheresis is graded in figure 2: "good" if the postapheresis platelet count was less than 500,000/µl; "moderate" if less than 750,000 but greater than 500,000; "poor" if less than 1,000,000 but greater than 750,000; and "uncontrolled" if the postapheresis platelet count was greater than 1,000,000/µl. The procedures resulting in "uncontrolled" platelet counts were in the first patient with the highest platelet count (greater than 4,000,000/µl), and in another patient in whom inadequate vascular access was obtained.

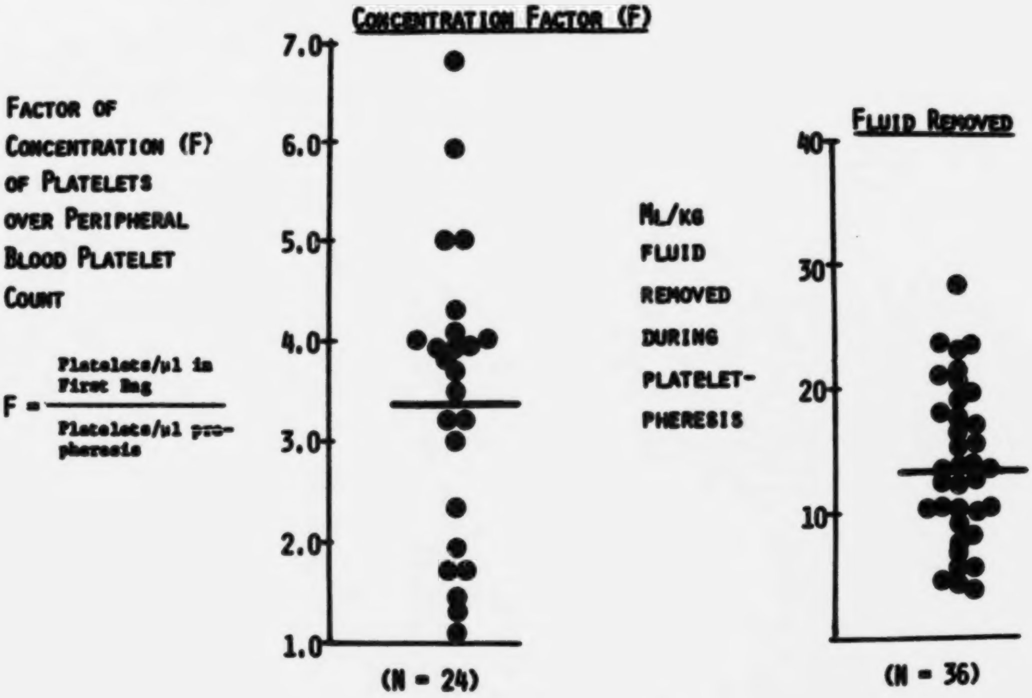
Figure 2.-- Decrements in platelet count for 41 procedures in 18 patients are shown, arranged from highest to lowest postapheresis platelet counts. The scale of platelet counts is linear from 0 to 1,000,000/µl, and logarithmic above 1,000,000/µl.



The yields (platelets removed from the patient) varied considerably, depending on the amount of clumping in the collection bag. In patients with thrombocythemia, clumping of platelets in the WBC tubing and collection bag is not uncommon, particularly if WBC ports are permitted to penetrate deep into the platelet layer near the "top" of the RBC band, and the rate of platelet removal minimized to obtain a high degree of platelet concentration. The rotational-stationary interface of the continuous-flow centrifuge appears to contribute to the clumping, and because the supernatant plasma will stimulate platelet aggregation of patient's platelets (taken after definitive medical reduction of platelet counts to normal), the supernatant is not returned to the patient. No adverse clinical effects have been noted in any patients.

The degree of concentration of platelets in the collection bag (i.e., number of times collected platelets have been concentrated over preapheresis peripheral blood) has varied considerably, averaging slightly less than 3.4 (see figure 3). A concomitant of the degree of concentration is the volume of fluid removed from the patient, also shown in figure 3. Expressed as ml/kg body weight, the highest losses approach the figures for small plasma exchanges and require appropriate fluid replacement.

Figure 3.--Factor of concentration of platelets (over initial peripheral blood concentration) achieved in 24 procedures and total fluid removed in 36 procedures are shown. The horizontal lines indicate mean values for each.

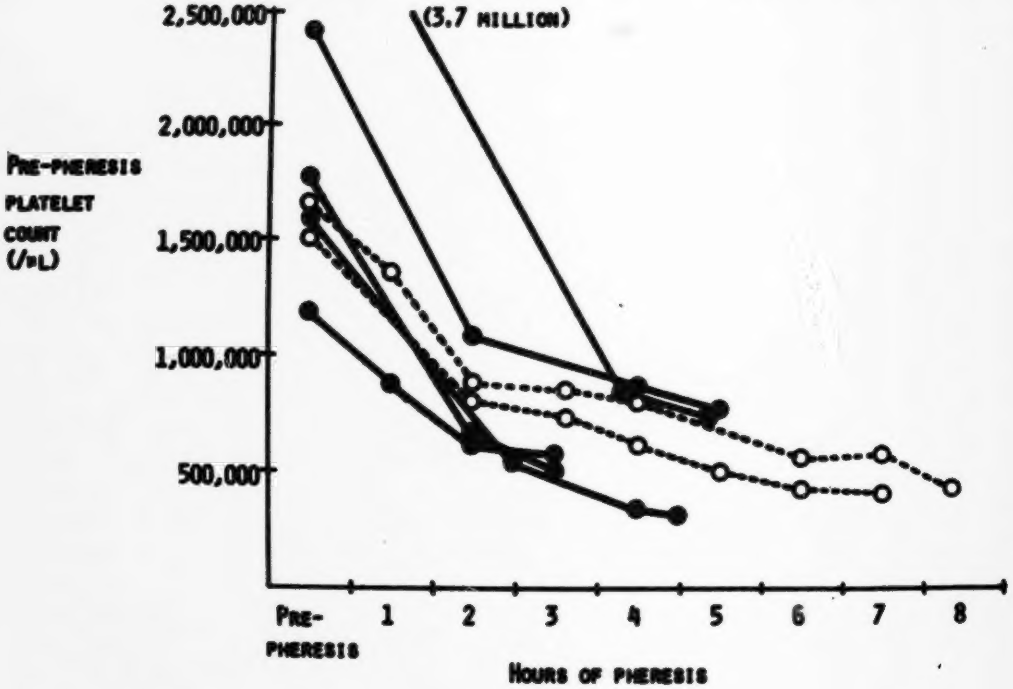


DISCUSSION

Plateletapheresis is a useful ancillary procedure in the management of patients with thrombocythemia. Current blood cell separators can lower platelet counts over several hours, given adequate vascular access. For successful management, however, it is imperative that appropriate chemotherapy and/or radioisotopic therapy be instituted as soon as possible, and be maintained so as to prevent recurrence of uncontrolled thrombocythemia.

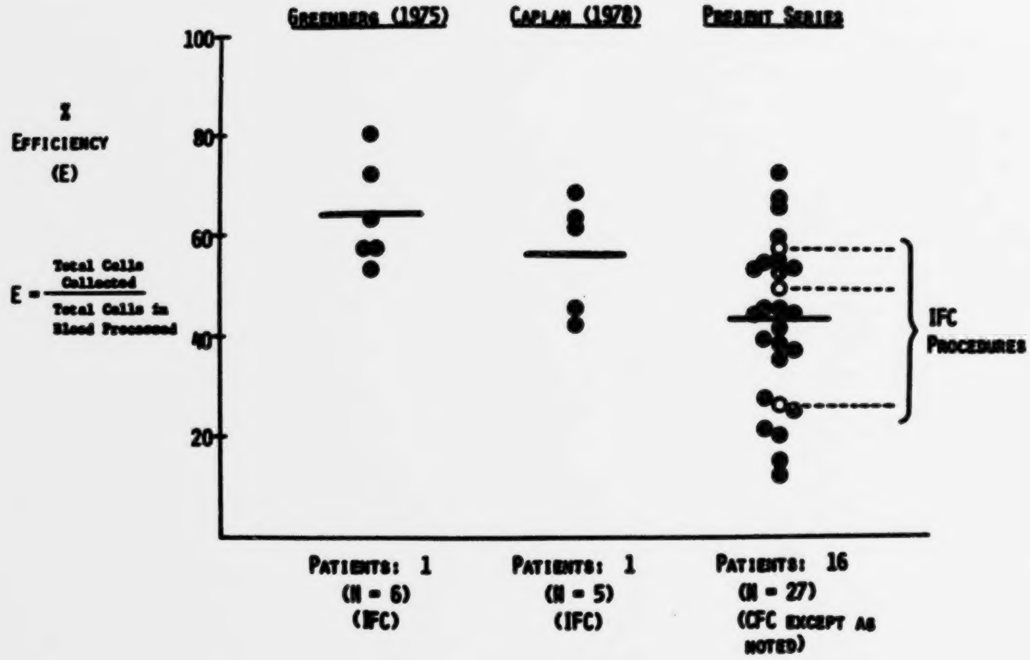
In the reports of therapeutic plateletapheresis in the literature, there are few measures of comparison of treatments among different patients. In figure 4, the progress of platelet decrement during plateletapheresis in five of our patients is compared to two (patients 2 and 3) of Colman's patients. In all patients, the greatest decrement occurs in the first few hours, and with extended-duration plateletapheresis, the decrements become progressively less. It would appear that centrifuge systems have made plateletapheresis easier, can process more blood per unit time, and may be better suited for patients with platelet counts in excess of 1,500,000/µl, but that the separation characteristics are similar to those of manual technics.

Figure 4.--This figure shows the pattern of platelet decrement for five of our patients (●——●) compared to two of Colman's (2) patients (patients number 2 and 3) during plateletapheresis.



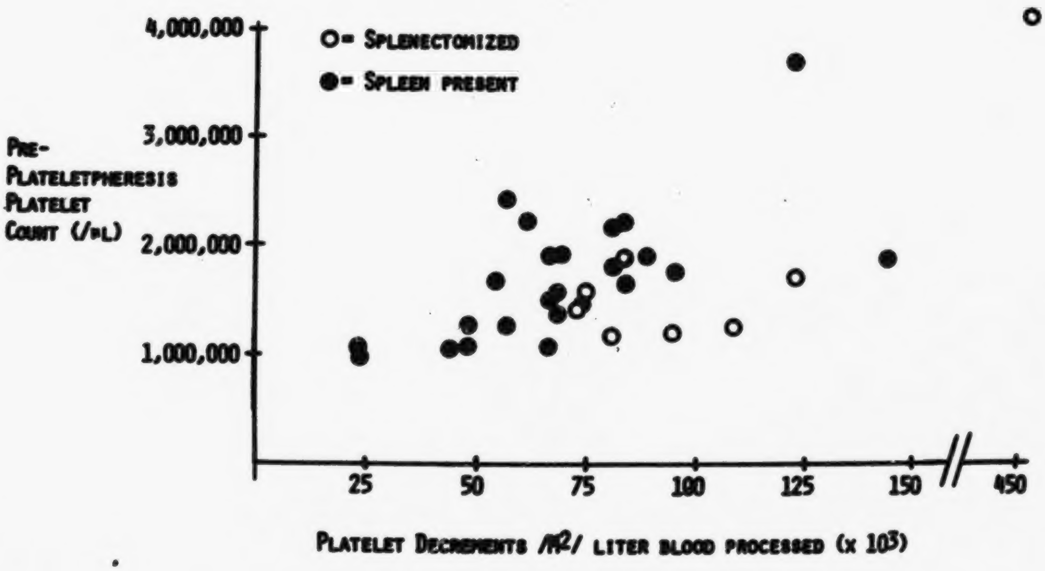
"Efficiency," an expression of the ability of plateletapheresis to separate platelets from other blood elements as presented from peripheral blood, has been used by Greenberg and Watson-Williams (5) and Caplan et al. (1) to compare multiple plateletaphereses in individual patients. Figure 5 compares their efficiencies with 27 procedures in 16 patients of this series. While there is considerable variation among the present series, the calculation of efficiency depends upon a reliable count of collected platelets. As indicated earlier, plateletapheresis by continuous-flow centrifugation has resulted in clumping, and therefore the counts from which "efficiency" is calculated must be considered minimum values. There is still another variable that makes comparison of efficiency difficult between continuous-flow and intermittent-flow centrifugation procedures, namely, that collection during IFC procedures is intermittent.

Figure 5.--Comparison of efficiencies of plateletapheresis from Greenberg and Watson-Williams, Caplan et al., and the present series. "Efficiency" is a function of reliability of counts on collected platelets (decreased by clumping), and does not reflect the actual outcome of plateletapheresis therapy (i.e., the magnitude of decrement). The horizontal lines indicate mean values.



Because there may be marked differences in the magnitude of circulating platelet pools in different patients (our patients varied from 27 kg to 97 kg) we have compared the patients and procedures using the decrement in platelet count/M^2/liter of blood processed. This reflects the decrement in platelets corrected for the size of the patient, and also the volume of blood processed. Figure 6 shows the decrement/M^2/liter against the initial (pheresis) platelet count. Splenectomized patients tend to have larger decrements than nonsplenectomized patients, presumably reflecting a smaller platelet pool volume, and more rapid (or more "efficient") reduction per volume of blood processed.

Figure 6.--Platelet decrements/M^2 body surface area/liter of blood processed plotted against preplateletapheresis count. Splenectomized patients (O) appear to have slightly greater decrements relative to their initial platelet counts, probably reflecting a smaller volume of distribution and/or less sequestration.



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Although the management of thrombocythemia has been simplified by application of apheresis, several problems remain. Additional experience is needed in patient selection. Symptomatic patients should be treated urgently, but indications for treating asymptomatic patients with myeloproliferative disease and platelet counts in excess of 1,000,000/μl are less clear, as they are in patients with reactive thrombocytosis. More reliable predictive tests may help define the patients most likely to suffer morbidity without plateletapheresis.

Vascular access remains a technical problem; the adequacy of peripheral veins tends to vary inversely with the urgency with which the patient requires treatment. Our cumulative experience now favors femoral venous catheters in place of A-V shunts when peripheral veins are insufficient.

Finally, next-generation centrifuges may offer improved separation and collection. While the plasma returned to the patient tends to be platelet-poor (less than 50,000/μl), the red cells may contain 100,000 to 200,000 platelets/μl. Undoubtedly this varies from patient to patient, but if returning red cells could be made more platelet-poor, then the procedure might be shortened slightly. Centrifuges bypassing the rotational-stationary interface principal may permit collection of platelets with better preserved functional properties.

We have found plateletapheresis a useful procedure in the management of symptomatic thrombocythemia. We look forward to the development of new blood cell separators and the appearance of additional reports describing the experience of other centers.

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DISCUSSION

Dr. Pineda: Thank you, Dr. Taft, for a very interesting presentation. Dr. Taft's paper is open for discussion.

Dr. Khan: Patients with myeloproliferative disorders usually have large-sized platelets, as compared to the ordinary platelet. I wonder if you have to adjust the speed of the spin, or make any adjustments to account for it. Because of their larger size, they may settle at a different layer, and do you take different cuts? Do you look for such changes, and do they account for some of the differences in the results obtained?

Dr. Taft: They may account for some of the differences. Really, plateletapheresis with the continuous-flow centrifuge, we find, is a fairly easy procedure, and you get a very distinct platelet layer with no problem at all [if you] set it at 1,500 rpm.

As long as we have a good layer there, we will just cut into it and reduce its size. We have not gone beyond 5 hours in any patient, or not had to go beyond 5 hours. If it were required to go 8, 9, 10, 11 hours, then [there would be] more incentive to look a little bit harder and then see what is going on in the red cell layer, ... the size of the platelets .... We just haven't had the incentive to look into that.

Dr. Nusbacher: What do you do with the platelets you collect?

Dr. Taft: Most of them have been thrown away, and recently they have been used for platelet freezing experiments.

Dr. Nusbacher: Do you know if they work?

Dr. Taft: Well, ... we get some clumped ones; ... we are just worried about getting the patient's platelet count down. Our own regional center supplies us quite well with platelets, and we have not had a shortage, so we have not used them, no.

Dr. Melanie Kennedy: A technical question about the use of catheters, particularly femoral catheters: I have had considerable difficulty using the type of soft-wall catheters that are used in CVP lines and cut-down lines, and you get very poor flow rates, even though you can show that your line is completely open by infusion. And I am wondering what type of catheter you use. Is it an angiocath?

Dr. Taft: This is what they call a Shaldon catheter, and I think the dialysis people can probably point you toward it. I don't do the procedure myself; the renal people are much more experienced than I am, so I let them do it. With those catheters, we get good flow, and at the conclusion of the procedure, we go ahead and remove it.

Dr. Kennedy: Yes. Those are not the soft-wall, and I just wondered--

Dr. Taft: No.

Dr. Kennedy: --if anyone else had had difficulty with soft-wall catheters.

Dr. Taft: There is a potential complication with the relatively rigid catheters, and we have seen one perforation of a femoral vein.

Dr. Gilbert Thompson: Do you measure whole blood viscosity? Do large numbers of platelets influence same, and if so, does it improve when you plateletapherese patients?

Dr. Taft: No, we don't measure whole blood viscosity, unfortunately. We have no data on that.

Dr. Thompson: And you don't know whether there is a tendency to hyperviscosity in thrombocythemia? Are there data on this?

Dr. Taft: I don't know of any data offhand. The maximum, the platelet "crit," generally isn't all that high, more than maybe about 10 mm or so, even in fairly high platelet counts.

Dr. Goldfinger: There appears to be a great reluctance, when performing apheresis, to remove anything but the component that is abnormal. This doesn't seem to make a lot of sense. In the case of treating acute leukemia or some other situation where there might be thrombocytopenia, we are very concerned that we are going to remove platelets and worsen the thrombocytopenia. However, 3 days later, the chemotherapist will give huge doses of chemotherapy, knowing that he will make the patient profoundly thrombocytopenic and will manage this with platelet transfusion.

We know from collection of platelets from normal donors that unless we take a significant number of red cells, that we get very poor efficiency of collection. And I wonder why we are reluctant to dig into that red cell layer a little bit, and if this requires giving the patient an occasional transfusion, that is really a small price to pay.

Dr. Taft: We don't like to dig into the red cell layer too much because we get a lot more clumping; maybe somebody else has had some experience with this. This seems to be a function of the concentration factor and also the rotational stationary interface. I think it is probably a technical problem more than anything else.

We are not averse, if the patients require transfusion, to give it to them. We would like to get out of plateletapheresis with as few procedures as possible, so we like to do each as [quickly as possible] and take the platelet count down as low as possible. And we don't like to see the clinician stalled in giving the chemotherapy, or whatever he is going to do, for definitive medical control. So we try to encourage them to get at it as soon as possible, and then we can get away with as few procedures as possible.

I think the clumping..., more than anything else, [deters us] from digging into the red cell layer, for most of these patients.

Dr. Goldfinger: I wonder if, in respect to the clumping, if you have tried using rather large volumes of ACD in the machine input line, and whether the patients that you have treated have been on antiplatelet drugs at the time that you start treating them.

Dr. Taft: Yes. Most of the patients have been on antiplatelet drugs. We have pumped in very high quantities of ACD and still gotten clumping. We have even put ACD in the lube and that makes everything sticky.

Dr. Grossman: Have you been using heparin in your input line? Because heparin by itself will act as a potent platelet aggregator in this situation.

Dr. Taft: Yes. We can cut that down to low levels, and just run ACD alone and still get [clumping].

Voice: Have you pheresed any patients after receiving P-32? [If so], how do you get the radioactive material out of the reusable Aminco bowl?

Dr. Taft: This is potentially a problem. On the other hand, the half-life of P-32 is very short, so this is the safety factor, I guess, if any were there. We like to take the platelet count down first, then have them give the P-32 immediately postapheresis. We like hydroxyurea a lot, for its safety factor, too.

Voice: Dr. Taft, just to follow up the point that Dr. Goldfinger made, what kind of red cell loss do you have in a continuous flow? It should be pretty minimal, I think.

Dr. Taft: Yes, it is really quite minimal; probably for each procedure, we are talking of maybe 20 or 30 cc of packed red cells.

Voice: We are using the semicontinuous-flow centrifugation system and, using hydroxyethyl starch, get very nice separation just by sedimentation of the platelets away from the red cells. And, in fact, if you sampled the red cell layer, it is virtually free of platelets and can be given back to the patient, if you wish to do so.

Dr. Taft: I can show you a couple of products and see what they look like, and this will give you an idea of some of the red cell contamination that we have taken, and also give you sizes of some of the platelet "crits," also.

[Slide]

Dr. Taft: I would say there is relatively minimal red cell loss here. This is the platelet crit here; I think this one is up a little bit higher in the bag; but it is relatively red cell free. This slide was taken after the red cells have been allowed to sediment.

Dr. Pineda: Thank you, Dr. Taft. ...

PLASMAPHERESIS IN THE TREATMENT OF HYPERVISCOSITY SYNDROMES AND PARAPROTEINEMIA

Jacob Nusbacher, M.D.
American Red Cross Blood Services, Rochester Region
University of Rochester School of Medicine
Rochester, New York

INTRODUCTION

Paraproteinemic states are characterized by the presence of qualitative and quantitative alterations in serum immunoglobulins. Undoubtedly many of the symptoms and pathological changes occurring in these conditions result from the presence of such proteins. In some cases, the pathological protein has certain physicochemical properties that induce symptoms directly, such as in patients with cold-precipitating globulins. In others, major manifestations of the disease result from blood hyperviscosity. This usually occurs in patients with macroglobulinemia where the primary pathological protein is in the IgM class. In multiple myeloma, however, despite elevated levels of immunoglobulins, hyperviscosity is not usual but occurs if the physicochemical properties of the paraprotein favor aggregation (4). In these conditions the pathological protein is usually in the IgG or IgA class.

Patients with paraproteinemic conditions often can be treated successfully with chemotherapeutic agents that destroy the abnormal protein-producing cell resulting in a reduction of the blood paraprotein levels. However, during the period of therapeutic induction, which may take a number of weeks, these patients are at risk of developing symptoms as a result of the presence of the paraprotein. In conditions associated with hyperviscosity, thrombosis and/or hemorrhage may result. Furthermore, some patients are refractory to chemotherapy. Thus, removal of pathological protein is an important therapeutic tool in these conditions.

Previous investigators have performed plasma exchange procedures on patients with hyperviscosity due to macroglobulinemia or multiple myeloma using routine batch plasmapheresis methods (3,4,5,10,11). While these methods are rather cumbersome and do not permit the exchange of large volumes of plasma within a relatively short period of time, there has been demonstrated a reduction in paraprotein levels and viscosity after such procedures. Plasma exchanges performed using automated equipment permit the removal of large volumes of patient plasma within a very brief time, thereby achieving a rapid therapeutic result which can be repeated periodically if necessary (8,9).

The purpose of this paper is to review our experience with plasma exchange, and to point out some important technical considerations relevant to these procedures.

TECHNICAL CONSIDERATIONS

Materials and Methods

Plasma exchange procedures were performed with either the Haemonetics Model 30 blood processor or the Aminco Celltrifuge. Both machines performed well, and only one procedure had to be terminated prematurely because of obstruction at the venipuncture sites. For all procedures, the exchange material consisted of albumin or plasma protein fraction (PPF) and 0.9 percent NaCl solution. In most cases, about two-thirds of the volume exchanged was replaced with colloid and one-third with crystalloid. Anticoagulation with the Haemonetics Model 30 was accomplished with a continuous infusion of ACD Formula B at a rate of 1 ml of anticoagulant for every 8 ml of whole blood processed. For Aminco procedures, in most cases, a single dose of heparin, 2,500 units, was given by a continuous infusion of ACD Formula B at a rate of 1 ml for every 20 ml of whole blood processed. This latter protocol reduced the risk of citrate-related symptoms.

The goal in all procedures was to accomplish an exchange of one plasma volume. The patient's blood volume was estimated from a nomogram based on height and weight. The plasma volume was then calculated by use of a venous hematocrit determination. It was recognized that these calculations were only approximations because many patients with hyperviscosity syndromes have an expanded plasma volume.

Kinetics of Plasma Exchange

The kinetics for any exchange transfusion, or for a plasma exchange, have been well delineated and can be described mathematically (2). It matters little whether the exchange is performed in a continuous-flow manner or by a conventional batch technique; the kinetics are essentially the same (figure 1). The shape of the exchange curve indicates that the greatest efficiency in removing pathological plasma constituents occurs early in the exchange, while later in the procedure, much less additional removal is achieved relative to the volume exchanged. Therefore, in the usual patient undergoing these procedures, exchange of one plasma volume usually suffices and will accomplish a 65 percent reduction in the levels of endogenous patient plasma. Table 1 gives the degree of exchange achieved at different exchange volumes.

Figure 1.--Kinetic curve for exchange transfusion (or plasma exchange) for both incremental and continuous exchange (2).

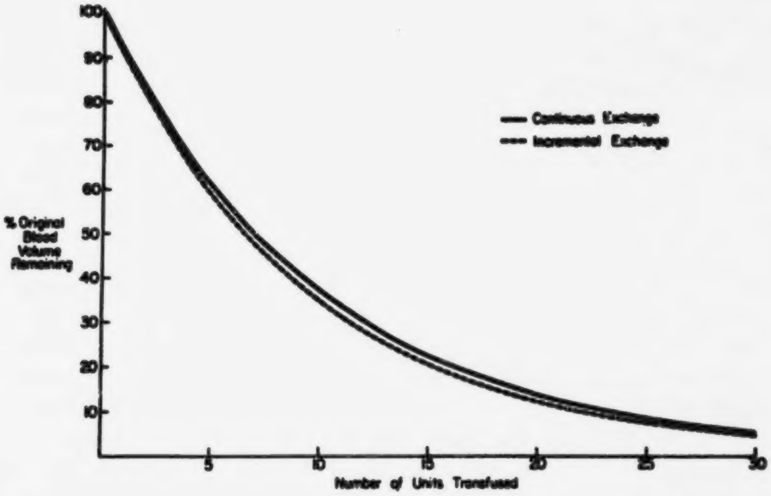


Table 1.--Relationship between volume exchanged and degree of exchange achieved

Plasma Volume Exchanged	Percentage of Original Plasma Remaining*
1	36.8 (34.9)
2	13.5 (12.2)
3	5 (4.2)

* Data are for continuous-flow exchange. Data in ( ) for incremental exchange.

These considerations apply on the assumption that there is no mobilization or synthesis of plasma constituents from extravascular sites. This is usually not the case. For example, assays of soluble clotting factors indicate that there must be significant and rapid replenishment of the vascular space during an exchange (1,6). While modest decrements in these factors are often noted, they are not commensurate with the calculated degree of exchange. With regard to immunoglobulins, it is well known that there exists a major extravascular pool of the IgG amounting to two-thirds of the total body pool and which presumably would be easily mobilized as the intravascular level falls. For IgM, on the other hand, 70 percent of the total body pool is intravascular, and intravascular reconstitution after exchange must rely primarily on new synthesis. Despite these considerations, when performing plasma exchange even for IgG multiple myeloma, there is little evidence that mobilization into the bloodstream occurs with such magnitude or rapidity as to justify using massive exchange volumes. Far more effective removal of pathological protein can be accomplished by sequential exchanges using smaller exchange volumes.

The kinetics of plasma exchange also mandates that effective replacement of plasma constituents is best achieved by infusion late in the exchange procedure. For example, in the patient with known deficiency in plasma clotting factors, plasma infusion should be reserved for the end of the exchange, while earlier in the procedure, "inert" materials such as albumin solutions or crystalloids may be used.

Viscosity Measurements

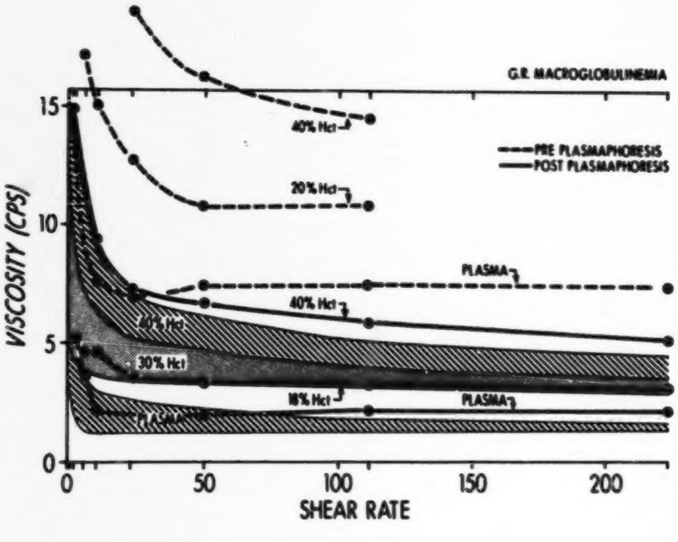
The viscosity of human blood varies with the shear stress and shear rate. This can be expressed in the following formula:

$$\text{viscosity} = \frac{\text{shear stress}}{\text{shear rate}}.$$

Thus, when measuring the viscosity of human blood *in vitro*, at any given shear stress the viscosity will be inversely related to shear rate. In physiological terms, measurement of viscosity at various shear rates is valuable because the effect of hyperviscosity at high shear rates, such as what may exist in the largest blood vessels such as the aorta, is not as great as that which occurs at lower shear rates existing in the microcirculation, the "business" end of the circulatory system. Furthermore, viscosity also varies considerably with the patient's hematocrit, and this must be taken into account when measuring whole blood viscosity.

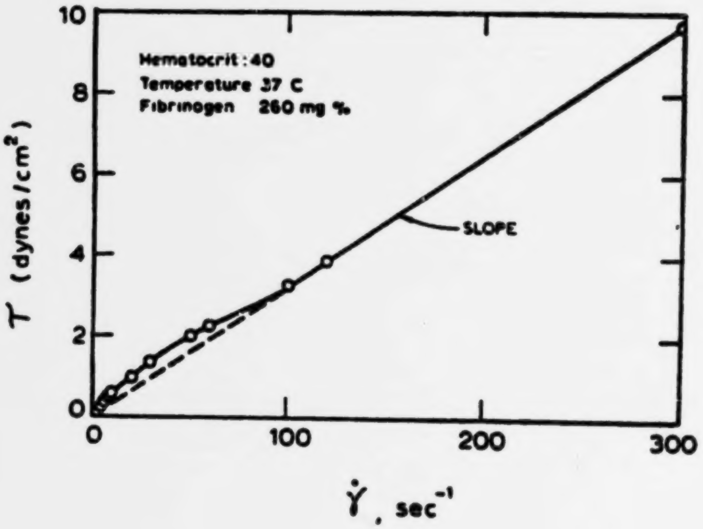
In our laboratory, we measure both plasma and whole-blood viscosity at a wide range of shear rates using a Wells-Brookfield cone-on-plate viscometer. If the patient is anemic, an additional viscosity measurement is taken after adjusting the whole blood hematocrit to normal levels. Figure 2 illustrates the viscosity curves obtained before and after a plasma exchange (of one estimated plasma volume) performed on a patient with macroglobulinemia. Prior to exchange, the patient's plasma viscosity was significantly higher than normal levels for whole blood. As this patient was also severely anemic (hematocrit of 20%) and required blood transfusion, if he were to be transfused without prior reduction of viscosity, the resulting whole-blood viscosity would increase even more and put the patient at even greater risk of developing hemorrhagic or thrombotic complications. The figure also shows the patient's viscosity curves after plasma exchange. While viscosity was not reduced to normal, the reduction was significant enough so that the patient could be transfused with a greater measure of safety.

Figure 2.--Viscosity curves obtained before (broken line) and after (solid line) plasma exchange in a patient with macroglobulinemia. Data are for plasma viscosity, whole- blood viscosity at the patient's hematocrit, and whole-blood viscosity at a normal hematocrit. Shaded areas represent range of normal viscosity for whole blood (at 40% and 30% hematocrit) and plasma.



To facilitate the evaluation of a patient over a long period of time and to compare the effects of repeated plasma exchanges, a minimum average viscosity (MAV) can be derived from the viscosity curves (7). Using viscosity measurements such as shown in figure 2, the shear stress at each viscosity is calculated using the formula shown above. If one then plots the square root of a shear stress against the square root of the shear rate, a straight line can be obtained over a relatively large range of values. (The slope of this line changes slightly at low shear rates but for practical purposes this does not appear too significant.) The slope of this line is the MAV (figure 3).

Figure 3.--Relationship of shear rate and shear stress to viscosity (MAV). Adapted from Merrill (7).



Viscosity measurements are a more meaningful reflection of the patient's pathological process and the effects of plasma exchange than are measurements of serum protein levels. It is well recognized that absolute protein levels are not necessarily related to the blood viscosity and, in some patients with very high paraprotein levels, viscosity as measured in vitro is perfectly normal. Therefore, the desired therapeutic effect of plasma exchange, which is a reduction in viscosity, can best be ascertained by viscosity measurements.

CLINICAL DATA

Since 1973 we have performed 61 plasma exchange procedures on 15 different individuals (range: 1 to 15 procedures per patient). The mean volume exchanged per procedure was 3 liters. Relevant clinical data on these patients are shown in table 2. Ten patients had Waldenstrom's macroglobulinemia; five had multiple myeloma (four of the IgG type, one of the IgA type). All patients were anemic (hematocrit range 17.5% to 34.0%).

In general, the patients treated by plasma exchange can be divided into two groups: those who required immediate reduction in viscosity primarily because of the presence of significant signs and/or symptoms; and those who were relatively asymptomatic. In the latter group, many of the patients were either unresponsive or no longer responsive to conventional chemotherapy. In the former group, retinal hemorrhages and associated visual disturbances were frequent (patients 6, 7, and 11). Other symptoms that required intervention were gastrointestinal bleeding (patients 9 and 10), neurological abnormalities (patients 5 and 7), and peripheral vascular ischemia with associated cold agglutinin disease (patient 8). One patient (number 13) required reduction in hyperviscosity preparatory to prostate surgery.

Viscosity Measurements

There was a general correlation between elevation of plasma protein level and the degree of hyperviscosity in both the macroglobulinemic and myeloma patients although there was much variation within the two groups (data not shown). Patients with macroglobulinemia generally had a greater elevation in MAV relative to other plasma protein level than did patients with myeloma and, for any individual patient, there was good correlation between elevated plasma protein and elevated MAV. These factors are illustrated in figure 4, which compares the total protein and MAV determinations in two patients, one with macroglobulinemia, the other with multiple myeloma.

Figures 5 and 6 show the viscosity measurements obtained after a series of exchanges in these two patients. It is apparent that a whole blood MAV can be brought to normal after a single exchange of one plasma volume, and that the rate of return to baseline protein and viscosity elevations varied between these patients. The data in the total group of patients are inadequate for drawing any general conclusions about frequency of exchanges necessary to maintain normal viscosity. Undoubtedly, variables such as paraprotein synthetic rate, stage of the disease, the total body pool of paraprotein and efficacy of chemotherapy all played roles in determining the rate of postexchange "rebound" among these patients.

Table 2.--Summary of clinical data on patients with paraproteinemia treated with plasma exchange

Para-protein	Number of Exchanges	Highest MAV*	Highest T.P.**	Initial Hct. (Percentage)	Equipment Used#	Clinical Findings
IgM	1	6.9	11.1	25	A	---
IgM	2	5.2	8.3	34	A	---
IgG	1	4.3	—	24	H	chest pain, dyspnea
IgG	17	4.8	12.3	26	H	uncontrolled by chemotherapy
IgG	1	5.6	14.2	25	A	headache, transfusion need
IgM	15	10.1	13.3	20	6-H, 9-A	retinal hemorrhage, lethargy
IgM	6	2.4	6.2	31	5-H, 1-A	retinal hemorrhage, dizziness, arm numbness
IgM	4	5.7	9.6	34	A	cold agglutinin dis., peripheral ischemia
IgA	1	3.9	—	30	H	bleeding, abnormal platelet function
IgM	1	—	—	30	A	g.i. bleeding
IgM	5	7.2	12.7	27	A	retinal hemorrhage
IgG	2	5.6	5.6	31	A	thrombocytopenia uncontrolled by chemotherapy
IgM	2	—	9.7	30	A	required prostate surgery
IgM	1	6.5	10.5	26	A	rheumatoid arthritis
IgM	2	6.7	—	22	A	coagulation abnormalities, unresponsive to chemotherapy

* Mean Average Viscosity

** Total Protein

# H = Haemonetics Model 30

A = Aminco Celltrifuge

Figure 4.--Relationship of total protein to whole blood MAV in two patients, one with macroglobulinemia (circles), the other with multiple myeloma (squares).

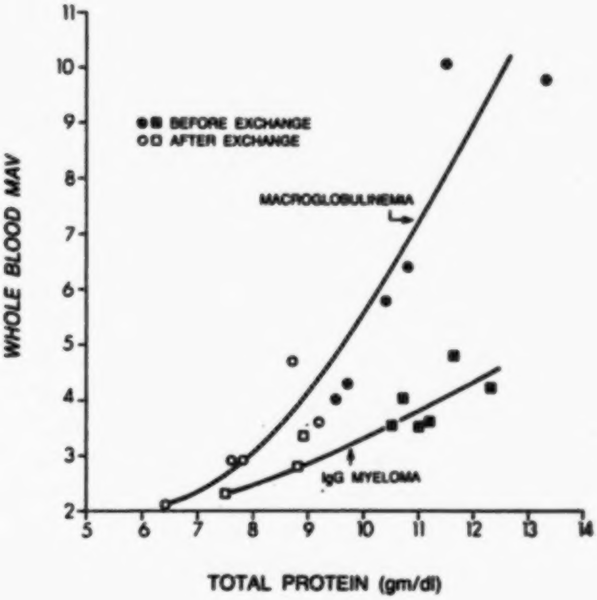


Figure 5.--Serial whole blood MAV measurements in a patient with macroglobulinemia (patient's hematocrit-20%). Arrows indicate plasma exchange procedures. Dotted line indicates normal MAV at 45 percent hematocrit.

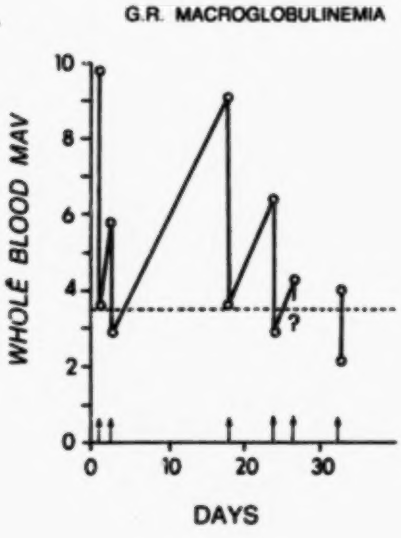


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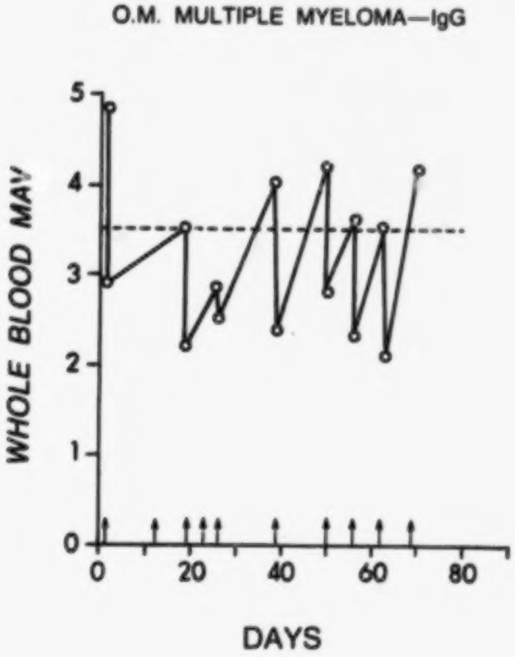
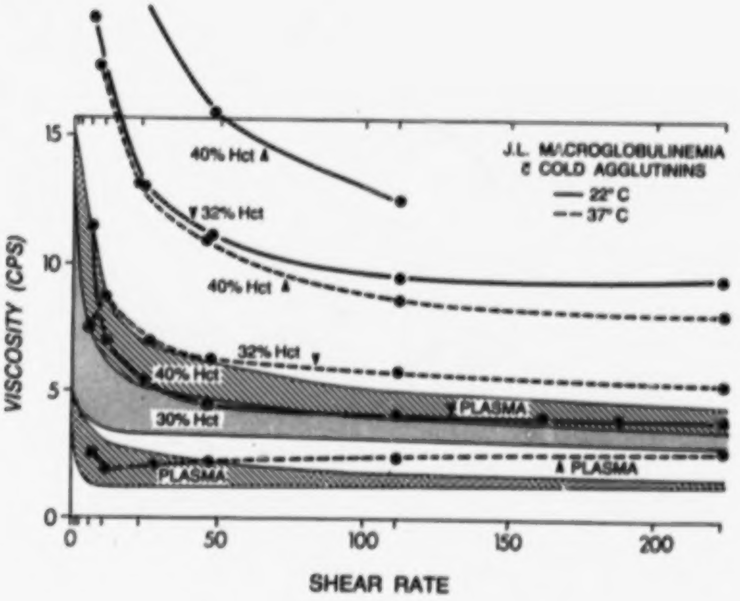
Figure 6.--Serial whole blood MAV measurements in a patient with multiple myeloma (patient's hematocrit 20% to 25%). Arrows indicate plasma exchange procedures. Dotted line indicates normal MAV at 45 percent hematocrit.



One patient (number 8) is of particular interest. This man had a monoclonal IgM paraprotein and hyperviscosity. He also had cold agglutinin disease as manifested by the presence of an antibody of anti-I specificity with a titer at 5 °C of 1:16,384 which fell to 1:4,098 after one plasma exchange. There was little hemolysis evident, however. Plasma exchange was required because of severe peripheral ischemic changes characterized by early gangrenous changes in his fingers. The latter findings abated completely over a period of months with four plasma exchanges and chemotherapy. This patient also had an unusual paraprotein that manifested greater plasma viscosity when measured at 5 °C than when measured at 22 °C (figure 7). The concomitant presence of a high-titer cold agglutinin may have served to elevate the whole blood viscosity even more, particularly at lower temperatures. This patient may be similar to one previously reported (11). The patient's paraprotein is being further analyzed.

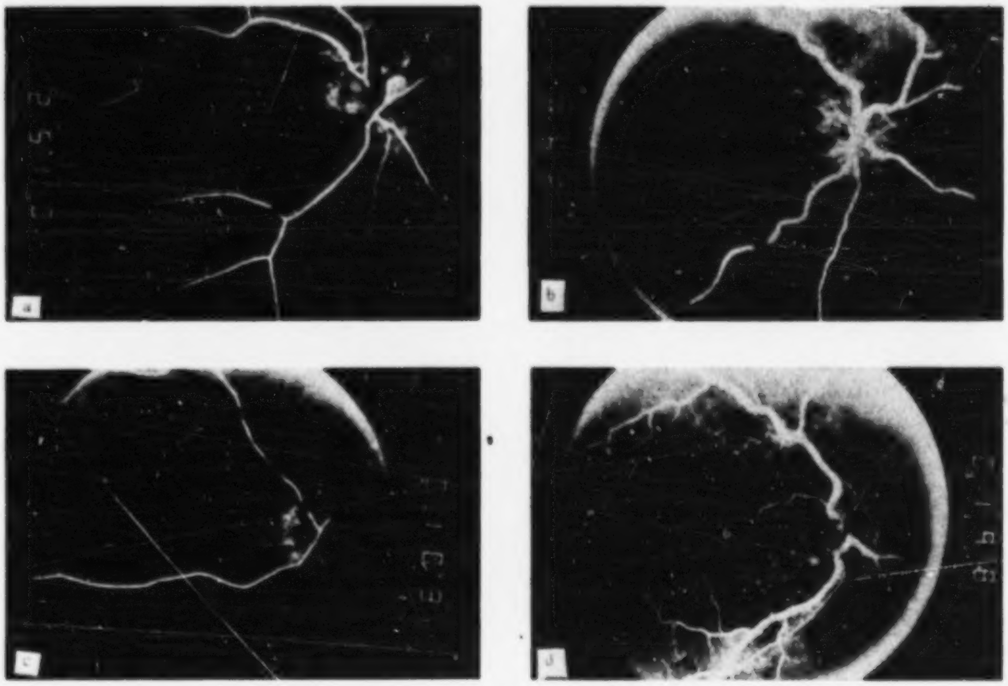
Figure 7.--Plasma and whole blood viscosity curves performed at 22 °C and 37 °C in a patient with macroglobulinemia. Shaded areas represent range of normal viscosity for whole blood (at 40% and 30% hematocrit) and plasma.



Measurements In Vivo

Improvement in flow in the microcirculation is the major goal in the use of plasma exchange to treat patients with hyperviscosity. A convenient method for following such patients is by measuring the circulation time in the retina using retinal fluorescein angiography (5). Figure 8 depicts fluorescein angiography performed before and after plasma exchange in a patient with macroglobulinemia. Before treatment, the time elapsed between maximum arteriolar filling and maximum venous filling was 14.6 seconds, and after exchange it was 9.5 seconds, indicating improved flow in the microcirculation.

Figure 8.--Fluorescein retinal angiography in a patient with macroglobulinemia. (a) maximal arteriolar filling, preexchange (b) maximum venous filling, preexchange (c) maximum arteriolar filling, postexchange (d) maximal venous filling, postexchange. Numbers indicate time (seconds) after intravenous injection of fluorescein.



DISCUSSION AND SUMMARY

Plasma exchange using automated equipment is an efficient and convenient method for reducing blood viscosity in patients with paraproteinemic states. In most cases, this procedure results in temporary benefit by alleviating symptoms resulting from hyperviscosity or, in the absence of symptoms, by reducing the potential for deleterious effects. Definitive treatment requires the use of chemotherapeutic agents directed against the paraprotein-producing cells. In some cases, patients will not respond to chemotherapy, and long-term plasma exchange is required to maintain blood viscosity within the normal range. Unfortunately, there are no randomized, controlled studies that would indicate the benefit of reduced (normal) plasma viscosity on the overall mortality in these patients. However, many case reports strongly suggest that serious sequelae of hyperviscosity can be interdicted by plasma exchange (3,4,5,8,9,10).

The measurement of blood viscosity in patients with paraproteinemias permits documentation of the presence of hyperviscosity as well as objective evaluation of efficacy of plasma exchange treatment. In most cases, measuring plasma viscosity suffices. However, the additional determination of whole blood viscosity permits a more realistic impression of rheological conditions because red blood cell concentration is a major contributor to blood viscosity. As patients with hyperviscosity are invariably anemic, the effect of an anticipated blood transfusion on viscosity can be more clearly judged with this approach. (See figure 2.)

Measurement of viscosity *in vivo* (figure 8) using fluorescein retinal angiography has been reported infrequently (5). This test is an easily applied tool for correlating paraprotein levels and *in vitro* viscosity measurements with blood flow alterations *in vivo*.

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DISCUSSION

Dr. Pineda: ... We invite you to comment or ask questions of Dr. Nusbacher.

Voice: I might have known Jack would get to it first. I had formerly inquired of various people to see if they had done...exchanges for cold agglutinin disease and couldn't find anybody.

I did one just yesterday, and this lady does have a cold agglutinin. Interestingly enough, I did see very minimal hemolysis; although it was apparent in the plasma bag, it was not in the tubes that I sent for titration, so I really don't know how much [it is going to help]. I did see a lot of precipitation in the bowl, but she tolerated the procedure quite well.

Dr. Nusbacher: It's very interesting, actually. The first time we did this, in my ignorance, we measured the cold agglutinin titer before and after exchange. The cold agglutinin titer was 1:500,000 before the exchange, and after it was 1:250,000. I was terribly disappointed, until I realized that we had reduced this antibody in half, and that is all you are going to do with an exchange.

Despite the fact that the kinetics are such that, if you have a high-titer cold agglutinin, it is really hard to get the titer down low where you might feel more comfortable about it, patients do seem to benefit.

And the other thing I would like to mention is that you ought to use a blood warmer on the blood going back to the patient, and the blood coming out, for that matter.

Voice: Well, certainly, especially when you have that much complement on the cells, already.

As you are saying, [our patient] did not show hemolysis *in vitro* when we did our thermal amplitude studies on her own cells, but she did on homologous cells; and I have also done a [patient with] cryoglobulin without cold agglutinin.

Dr. Taft: Jack, on your patient with the hematocrit of 20, did you do a red cell mass?

Dr. Nusbacher: No. He probably had a bigger red cell mass than indicated by a 20 hematocrit, but I don't think he had a red cell mass equivalent to a 40 percent hematocrit.

Dr. Taft: I think we ought to make this point. We have run across a couple of patients with very unimpressive plasma viscosities, but rather massive hypervolemia. And I am not sure that the hypervolemia isn't really the major defect in some of these patients, and the major thing we want to correct.

What we have done ever since our first plasmapheresis patient is to take off up to 3,500 ml at a single sitting, replacing it only with a little Ringer's: Most patients will tolerate this quite well. But you have to know how much excess plasma volume there is, so you don't get them hypovolemic.

Voice: How much did you take off? Tell me that again?

Dr. Taft: We have taken off up to 3,500 ml of plasma at a single sitting, in a patient who had a plasma volume of 3,500 ml, replacing it only with Ringer's solution. The viscosity comes down in the patients who are hyperviscous, but the plasma volume also shrinks down to normal.

Dr. Ries: I would like to reinforce that same concept. I think Feudenberg and Perkins showed that 10 years ago, when they actually measured blood volumes in a whole bunch of patients with myeloma. Some of these plasma volumes were 2 or 3 liters above normal; so much that you wouldn't expect that they could fit it into a circulatory volume. And I think the comment you made about some of your clinicians telling you that you can't have a hematocrit of 40 when you have a hyperviscosity--the reason for that is that you have such a markedly expanded plasma volume.

Dr. Nusbacher: Let me say something: There is evidence that marrow release of erythroid elements is also reduced in patients with hyperviscosity. So there may be more to it than...

Dr. Ries: Yes. Actually, almost always the anemias are mixed anemias. It is usually not just due to that. But when we used to do plasmapheresis by bags, while you are taking the first two units out to spin down, often the patients would say they felt remarkably better, even before you gave them their red cells back; so I think a lot of their symptoms are due to volume expansion.

There was one thing I wanted to ask you: Have you had any problems with citrate toxicity at the flow rates you have used, and at the levels of citrate you've used?

Dr. Nusbacher: No, we--well, I wouldn't say "no." We have become very blase about the little tingling around the mouth, and things like that.

The protocol we use for the Aminco--and we are using whatever flow rate the vein will tolerate, and it is usually slower at the beginning and faster at the end--the protocol we use consists of a small amount of heparin, 2,500 units, and a 1:20 ratio of citrate--or ACDB (which has somewhat less citrate) to whole blood. This almost completely eliminates the citrate problems.

When we are using the Haemonetics, we don't use that protocol. I don't know why, but we just don't. The reactions you see are what you would see in any plateletapheresis donor, or any donor on that machine. Usually you will see symptoms which are easily managed just by slowing the reinfusion rate.

Dr. Ries: Have you had any problems with the patients that have had myeloma, in terms of keeping the flow going even with a good-sized catheter in the vein?

Dr. Nusbacher: We have, and haven't. We are using large needles, primarily, or large catheters. With the Aminco, if you watch the procedure, with patients with a severe hyperviscosity, the pumps are working hard, and there is a lot of air and other things crossing over the seals because of the increased viscosity. Fortunately, all the air that is coming from the outside room is going into the plasma collection bag, so it is not going back to the patient. But the pumps work hard. ...we only had to terminate one procedure because of poor flow. Otherwise, we have been able to manage it.

Dr. P.K. Ray: I would like to make a comment in this connection: We had been working for some time to find out whether or not selective immunoadsorption of the abnormal protein, like IgM or IgG, is possible by using the Aminco cell separator and an immunoadsorption column. We have devised such a technique by which extracorporeal immunoadsorption of IgG can be done in animals and humans.

We have treated an IgG-type multiple myeloma patient, and the plasma which was separated by the Aminco centrifuge machine was passed through a capsulated bacterial membrane column containing heat-killed formalin stabilized *Staphylococcus aureus* suspension.

This particular bacterium contains protein A on its membrane; and protein A can selectively bind the IgG molecule. We have been able to separate out about 50 to 60 percent of IgG from the IgG-type multiple myeloma patient within 2 to 3 hours' time. ...a total protein which was 12.8 gram/percent in that particular patient...was brought down to the order of 5.6 after two such procedures. There was significant improvement in that patient. A patient who was not responding to chemotherapeutic drugs--her condition improved. Thus, we believe immunoadsorption procedure may be used in this disorder as an alternate method.

In an attempt to have a specific immunoadsorbent, as can be used in case of macroglobulinemia, we have been able to devise such a technique. In this case, Concanavalin A bound to polysaccharide beads had been used.

Dr. Nusbacher: Excuse me. Did you measure viscosity after removing [the IgG protein]?

Dr. Ray: Oh, sure. The viscosity of the IgG multiple myeloma patient before treatment was 3.69, and after two treatments, it came down to 2.8.

Dr. Nusbacher: I might say that in this particular condition, specific removal of paraprotein has some economic benefit. If you don't have to reinfuse any albumin, which is very expensive, you are far better off. There are probably many ways of doing that, but the way you have described is absolutely unique. I am sorry I didn't think of it.

Dr. Pineda: That particular field of interest is going to be discussed at great length tomorrow afternoon, when [affinity] columns are going to be the subject of discussion. And your column, I take it, is very similar to Dr. Bansal's and Terman's, with regard to the removal of IgG. And I am glad to see that you have gotten a step ahead of the game by being able to remove IgM, something that we didn't think we could do efficiently.

Dr. Herbert Polesky: Just to comment on the economic value of not having to use the albumin, we had a patient some years ago, before we had machines, with a cryoglobulin that was apheresed with a bag technique, for about 2 liters at a time. And we found that using the closed system by taking the plasma and putting it in the refrigerator overnight, for 2 days, we could keep that plasma and give him back his cryopoor plasma on the following visit. And that way, we [had] to use hardly any albumin or any other protein. We kept this guy for about 5 years with this technique, with very little substitution of anybody else's plasma.

Dr. Nusbacher: Yes. That is a good technique, by the way. Dr. Berkman has done that in Boston, and he has...published or he is going to be publishing a paper on some of his experiences with that.

Can I make a comment about viscosity. There were some questions about the relationship of leukostasis and viscosity measurements with high platelet counts: I am not aware of viscosity measurements with high platelet counts, but I am aware of studies on viscosity, whole blood viscosity, measurements with white cells. Lichtman and colleagues published that quite a while ago.

White cells, even leukemic cells, are not a major contributor to blood viscosity. To give you some frame of reference, you have to have about 2 million white cells per cubic millimeter to give you the same equivalent viscosity that you would get with a normal person with hematocrit 40 percent.

In other words, the leukostasis that you see, and I don't doubt for a moment that it exists in high-count leukemia, is not necessarily related to the fact that the blood has hyperviscosity, at least as you measure it in vitro. It may just be plugging.

Now, you can get into a semantic argument. However, when measuring viscosity by standard techniques in vitro, the white cells, and probably, I suspect, the platelets, are not major contributors to the viscosity. In normal blood, the major contributors to viscosity are red cells and fibrinogen.

Dr. Gilcher: I think that part of this relates to the deformability of cells.... As we know, the mature red cell is very deformable, but nucleated cells, specifically blasts, are very nondeformable cells. And that may be one of the very important mechanisms in leukostasis, ...nondeformability. So we can't talk about viscosity, because we are really at a capillary level in talking about the deformability of the cell.

Dr. Nusbacher: That is a good point. I asked Marshall Lichtman that just the other day...because he published studies on deformability of immature cells. And he said, "Maybe that's the reason."

Dr. Pineda: Any further comments or questions? Dr. Taswell has an announcement to make before we break for coffee.

Dr. Taswell: There have been a number of requests from people who want to take a tour of the [Mayo] clinic. I had mentioned a tour of the blood bank; if there is enough interest we can arrange for [both]. There is a regular clinic tour for the public.... There are also special tours for physicians, which I am sure you would find much more interesting. It could be arranged for this evening, after dinner, if there is enough interest.

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PLASMAPHERESIS IN THE TREATMENT OF IMMUNE DISEASE

Richard F. Branda, M.D.
J. Jeffrey McCullough, M.D.
Departments of Medicine and Laboratory Medicine
University of Minnesota
Minneapolis, Minnesota

Circulating antibodies have been implicated in the pathogenesis of many human diseases. Consequently, treatment of those diseases usually includes pharmacologic agents that alter immune function. While that form of therapy is often adequate, its usefulness is limited in some patients by the appearance of serious side effects and in others because a more rapid response is needed. An alternate therapeutic approach in such often critically ill patients is to remove or lower antibody levels by plasmapheresis. The approach seems promising, but it also has major limitations. In particular, its effective application is hindered by our incomplete understanding of disease mechanisms. In addition, in many clinical situations the kinetics of antibody production cannot be characterized because the inciting antigen is unknown, antibody assays are technically difficult and imprecise, and evaluation of therapeutic effect on antibody levels is complicated by the utilization of diverse forms of therapy. Those problems, as well as the potential value of plasmapheresis, are illustrated in patients with idiopathic (autoimmune) thrombocytopenic purpura (ITP).

Some cases of immune thrombocytopenia develop after a viral infection or drug therapy, but more often, particularly in adults, the initiating agent cannot be identified. However, it is known that the thrombocytopenia is mediated by a circulating antiplatelet factor, because infusion of plasma from ITP patients into normal volunteers precipitously lowers their platelet counts (11). Not all patient plasma has this thrombocytopenic effect, and subsequent studies with a variety of assays identified an antiplatelet antibody in the plasma of 50 to 80 percent of ITP patients (15). In the remaining patients, antibody appears to be largely bound to platelets (5). That this circulating antibody is pathogenic in ITP is further corroborated by the appearance of neonatal thrombocytopenia in babies born to mothers with ITP, presumably due to transplacental diffusion of the antibody

(9). Therefore, while the exact disease mechanism is poorly understood, inhibition of antibody production or enhancement of its clearance should be of benefit in patients with ITP. In practice, significant inhibition of ongoing antibody synthesis is difficult (17), but removal of antibody-producing cells by splenectomy (13) or modification of antibody-platelet interactions with steroids (7) usually leads to amelioration of ITP. Occasionally this conventional treatment is ineffective or dangerous, while in patients with fulminant disease a more rapid response may be necessary. In such difficult patients, exchange may be a useful adjunct to treatment.

For example, we have noted clinical improvement in two of four ITP patients following plasma exchange. The first was a 29-year-old housewife who received chlorthiazide for pedal edema during her first pregnancy (1). Two weeks later she saw her physician because of easy bruising, and her platelet count was found to be $49,000/mm^3$. Although her medication was immediately stopped, she continued to have severe thrombocytopenia. Evaluation showed increased megakaryocytes in her bone marrow and the presence of antiplatelet antibodies in her plasma. Prednisone in high doses was begun, but thrombocytopenia persisted and was accompanied by bleeding. Approximately 1 month after delivery of a normal infant she developed steroid psychosis, and that, plus marked obesity, was considered a contraindication to immediate splenectomy. After plasma exchange, her platelet count rose from $31,000/mm^3$ to $170,000/mm^3$. A transient decrease in platelet count 2 weeks later was followed by a permanent remission. Following plasma exchange, platelet antibodies were no longer detected.

The second case was a 21-year-old electrician who developed prolonged bleeding after tooth extractions (2). He was found to have severe thrombocytopenia and was begun on high doses of steroids, but the therapy was accompanied by gastrointestinal bleeding and hypertension. Following splenectomy he had a transient rise in platelet count, but then he developed severe intra-abdominal hemorrhage. Despite increases in prednisone dosage, vincristine therapy, and platelet transfusion, the hemorrhaging persisted, and reoperation was considered necessary. Instead, plasma exchange resulted in a prompt rise in platelet count and a stabilization of his hemoglobin level. Five days later his platelet count again dropped, necessitating a second exchange. An increase in platelet count to $120,000/mm^3$ occurred, followed by gradual stabilization at about $50,000/mm^3$. Subsequently his platelet count rose to normal levels. Antiplatelet antibody activity was present in the patient's plasma prior to plasma exchange, and the level dropped immediately after that procedure. At the time of his discharge from the hospital, increased antiplatelet antibody activity was no longer measurable.

The third case was a 25-year-old housewife who had a history of thrombocytopenia and gastrointestinal bleeding 12 years earlier (1).

Her platelet count returned to normal after 2 months of steroid therapy. One week prior to admission she developed a mild upper respiratory infection followed by petechiae and ecchymoses. Evaluation showed platelet counts of less than 20,000/mm^3 and increased numbers of megakaryocytes in the bone marrow. A trial of steroids was unsuccessful, and a single plasma exchange did not alter the platelet count. Splenectomy was followed by a prompt response.

The fourth case was a 26-year-old truck driver with a 20-year history of intermittent episodes of Coombs-positive hemolytic anemia, requiring splenectomy and high doses of steroids. On the last of his many admissions to our hospital he had severe hemolysis, with a hemoglobin of 4.7 gm percent, thrombocytopenia, reticulocytopenia, a hypoplastic bone marrow, thrombophlebitis, and evidence of pulmonary emboli. Because of progressive deterioration, despite massive doses of steroids, plasma exchange was performed. No improvement of either thrombocytopenia or hemolysis was noted, and the patient died 3 days later with pulmonary emboli and sepsis.

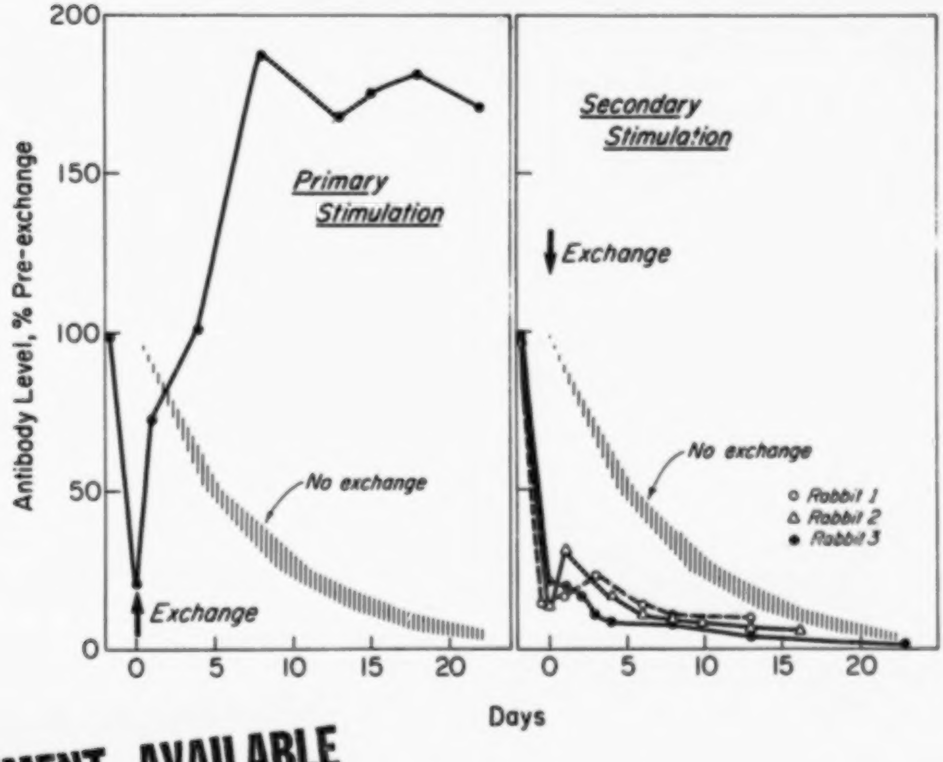
The reports above, and subsequent ones, suggest that plasmapheresis may be of benefit in perhaps one-third of ITP patients so treated (table 1). Since many cases have been described only briefly or in abstract form, it is difficult to discern clinical features that might predict the outcome of plasma exchange. It seems likely, however, that the range of clinical response reflects, at least in part, differences in the kinetics of antibody production. Unfortunately, in most cases of ITP the initiating antigen is unknown, and the kinetics of antibody production cannot be accurately determined.

Table 1.--Outcome of plasma exchange in ITP patients

Author	Cases Treated	Improvement	Reference Number
Branda	4	2	1,2
Isbister	4	2	12
Moulinier	11	4	14
Novak	1	1	16
Weir	1	0	18

Studies in animals suggest that the nature of the immune response critically influences the outcome of plasma exchange. Uhr and his coworkers noted that plasmapheresis of animals during their response to a primary antigenic stimulus led to rapid synthesis of new antibody, and that postexchange antibody levels exceeded preexchange levels by 48 to 222 percent (3). The rebound phenomenon is illustrated in figure 1, left panel. A rabbit was injected with bovine serum albumin (BSA), and exchange transfusion was performed 18 days later, when the antibody level was decreasing. The initial drop in antibody level was followed, within a day, by rapid reaccumulation of antibody, which eventually nearly doubled the preexchange level (1). In contrast, when animals were given exchange transfusions 7, 11, or 18 days after the second of two immunizations 30 days apart, antibody was permanently removed (figure 1, right panel). Although slight increases in antibody levels were seen after exchange transfusions (probably due to redistribution of tissue antibody), no rebound occurred in the animals with secondary stimulation of antibody production (1). The remaining antibody levels decreased at the rate expected in animals not given exchange transfusions.

Figure 1.—Effect of exchange transfusion on rabbit immune responses to BSA. Left: Exchange was performed 18 days after primary immunization and was followed by a rebound of antibody levels (closed circles). Right: Effect of exchange 7 days (closed circles), 11 days (open circles), or 18 days (triangles) after secondary immunization on antibody levels. Shaded areas in both panels represent antibody levels in unexchanged animals.



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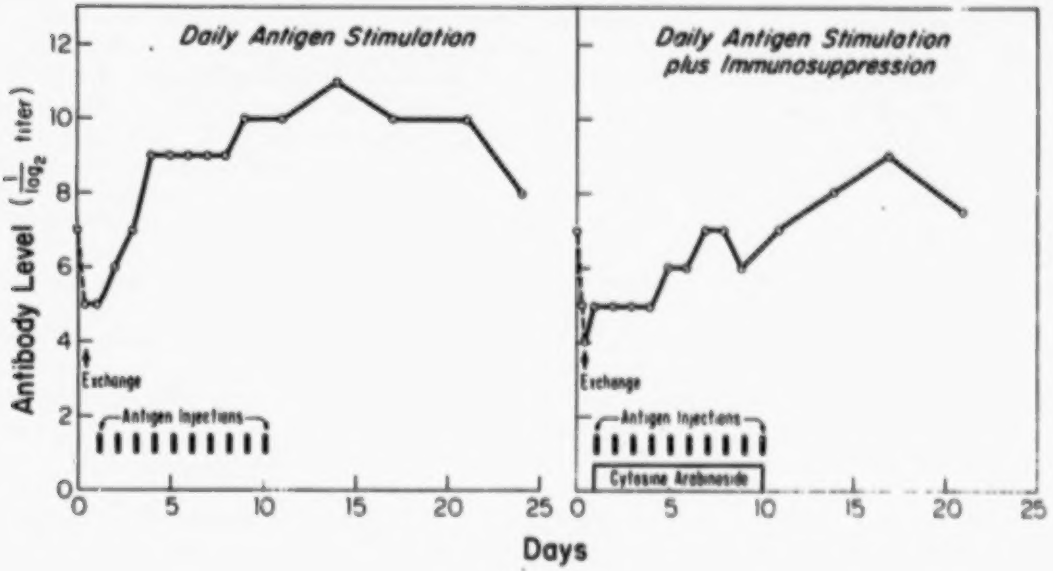
When antigen is injected more frequently, exchange produces only a temporary lowering of antibody levels. For example, a dog was repeatedly injected intravenously with sheep red blood cells (SRBC) in saline. Subsequently, plasma exchange, using a continuous flow centrifuge, was performed, followed by daily injections of SRBC. As shown in figure 2, left panel, plasma exchange effected a transient decrease in antibody levels (as measured by hemagglutination titers), but shortly afterward high levels of antibody reappeared. The experiment was then repeated in the same dog with the addition of cytosine arabinoside, 20 mg/kg/day, beginning the day of plasmapheresis (figure 2, right panel). Cytosine arabinoside was chosen because it is a cycle-active agent and is known to produce immunosuppression in dogs (10). Once again, plasma exchange lowered antibody levels, but the return of antibody was only partially modified by this immunosuppressive therapy when antigen was injected daily. Those animal studies suggest that the type of immune response and the frequency of antigenic stimulation determine, at least in part, the response of antibody levels to plasma exchange.

Figure 2.--Effect of plasma exchange on dog immune responses to sheep red blood cells. Left: Exchange during an anamnestic immune response was followed by daily injections of SRBC. Antibody levels (open circles) promptly rose above preexchange levels. Right: The same dog underwent a second plasma exchange, followed by daily injections of SRBC plus cytosine arabinoside. The return of antibody levels (open circles) was delayed.



The direct relevance of the animal studies to man is unclear, but some evidence suggests that they may be applicable. Plasmapheresis or exchange transfusion has occasionally been used clinically in situations where the antigenic stimulus is known. Analysis of those cases provides insights into antibody kinetics following exchange in humans.

For example, exchange transfusion has been used in the treatment of posttransfusion purpura. This rare disease occurs in individuals, usually women, who lack the PlA1 platelet antigen. Primary immunization occurs by exposure to the antigen during blood transfusions or pregnancy. If the patient is again exposed to the antigen (usually by transfusion), high levels of antiplatelet antibodies are produced, which results in severe thrombocytopenia. The antibody (and thrombocytopenia) can persist for as long as 6 weeks. Exchange transfusion, or plasmapheresis, promptly lowers the antibody levels, and the reduction is permanent (4). Simultaneously, the platelet count rises, effecting full recovery.

Another clinical situation where the antigenic stimulation can be identified is in hemophiliacs with Factor VIII antibodies. Cobcroft and colleagues (6) recently described one such patient who had a persistent Factor VIII antibody. Plasmapheresis, performed 4 weeks after an infusion of Factor VIII, when consecutive antibody levels were 4.5 and 4.0 units/ml, lowered the antibody level to 1.2 units/ml. The authors noted that after eight successive phereses the antibody level was the lowest recorded for 3 years, and no rebound of antibody was apparent.

In contrast, if Factor VIII is continuously administered, plasmapheresis is much less effective in lowering antibody levels. We studied a patient with severe hemophilia who developed a Factor VIII antibody at age 17 (8). Three years later he developed a large muscle hematoma. Plasmapheresis rapidly lowered an antibody level of 12 units/ml to undetectable levels and large quantities of Factor VIII replacement were given. Five days later an anamnestic response appeared, with production of very high levels (128 units/ml) of anti-Factor VIII antibody. Fortunately, healing of the hematoma was permitted by this short period of replacement therapy. Six months later, when his Factor VIII antibody level was 8 units/ml, he developed a subdural hematoma. Plasmapheresis was again performed, this time followed by therapy with steroids and Cytoxan. An anamnestic response occurred, as expected, after 5 days but was attenuated by the immunosuppressive therapy. However, when the drugs were stopped, high levels of antibody reappeared, necessitating a third plasma exchange. Thus, plasmapheresis plus immunosuppressive therapy can modify but apparently not ablate an anamnestic response in humans when large amounts of antigen are continuously present.

Taken together, the animal data and clinical observations reported here suggest the following tentative conclusions regarding the use of plasma exchange in immune diseases:

1. Plasma exchange during a primary immune response is followed by a prompt rebound of antibody.

2. Plasma exchange during a secondary response appears to remove antibody permanently.

3. Exchange during frequent or continuous antigenic exposure only temporarily removes antibody, but the return of antibody can be delayed and diminished by immunosuppressive drugs.

It is apparent that characterization of antiplatelet antibody levels and production in patients with ITP will be of great help in identifying those likely to benefit from plasma exchange. Certainly, circulating antibodies should be documented before exchange, because significant numbers of ITP patients have only platelet-bound antibody, and in such patients plasma exchange is unlikely to result in improvement. To the extent that the conclusions above are applicable to immune thrombocytopenia, we suggest that plasmapheresis will be most effective in patients with anamnestic immune responses without recurring antigenic stimulation, such as in posttransfusion purpura or following drug ingestion. In patients with chronic antibody production, plasmapheresis is likely to lower antibody levels only temporarily and to raise the platelet count. Nevertheless, such transient improvement may be clinically valuable to achieve hemostasis in actively bleeding patients. Before plasmapheresis is applied more widely in ITP patients, we hope that its effect on antibody levels and lymphocyte populations (particularly suppressor cells) is defined more clearly, and that a measure of its effectiveness is determined by controlled trials.

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DISCUSSION

Dr. Pineda: Thank you, Dr. Branda. Dr. Branda's paper is open for discussion.

Dr. David Terman: I was surprised to observe a suppression of circulating antibody when exchange was performed in the course of a secondary response.

Dr. Branda: There were two different studies. One was in rabbits, looking at primary and secondary stimulation; the second series of experiments was in dogs with daily injections of antigen. We were really looking at two different things. The rabbit studies were looking at only the effect of exchange on antibody levels. The dog studies were looking at the exchange in the presence of frequent antigenic stimulation and possible modification with immunosuppressive drugs.

Dr. Terman: ...the conclusion that you are leaving is that plasmapheresis may be effective in the course of an anamnestic response. It is conceivable that what you were doing is reducing the antigenemia with exchange, and hence lowering the antigenic load in the animals that may be capable of sustaining the secondary response.

Dr. Branda: If you are talking about the exchange of the anamnestic response, as I said, those studies were done in rabbits. We did six or eight rabbits. The animals received the primary immunization, a month later received the secondary immunization, and then, several days later--I don't remember the exact number--something like 7 to 18 days later--we exchanged the animals.

Now, that was at various points in the secondary immune response. As you know, the secondary immune response comes on sooner than the primary; usually by 4 or 5 days it can be detected. So we did some exchanges when it was a well-established secondary immune response, other animals at the peak of the secondary immune response, and the third group of animals when the response was on the way down. And in all cases, it appeared that the antibody levels did not rebound in the way that they did with primary immunization, in that we did not see a return to antibody levels higher than preexchange.

Dr. Terman: Hence it is conceivable that in the course of exchange, you were reducing the antigenic load, and without T 1/2's on the antigen, it is conceivable that you were inducing low-dose tolerance in the immunized animal.

I raise this point because the host in the course of an anamnestic response should be making a florid response and should replace the antibody that was removed from the plasma very rapidly.

Dr. Branda: Yes. I am aware of your studies, and I have naturally given them a fair amount of thought; but we are doing different things.

I think, if I can digress here for a minute, first of all, you are exchanging your dogs much later in the immune response than we are, if I read your papers correctly--somewhere at 3, 4, 5, 8, 12 weeks after the secondary immunization, isn't that correct? So, in other words, you are exchanging your animals much later in your immune response; and it is possible, at that point, there is a difference in what happens to the antibody. For one thing, the antibody levels would be lower. When we have looked at things like ASO titers in our patients who have not had any strep infection recently--in other words, these are titers from the distant past--and looked at titers pre- and postplasma exchange, we don't find much difference.

So it may be that the timing is critical in what the response is, here. That is one difference.

The second difference is that Patterson, 15 years ago, showed that dogs and rabbits are different in their formation of precipitating antibody to BSA, and there may be technical differences between our experiments.

The third thing that is different about our experiments is that, if I am again interpreting your papers correctly, your rebound occurs several days after the exchange, whereas in our primary animals, and in the cases reported by Bystryn and Uhr, rebound happens within a day. And I would worry, and I know you have thought about this, but I would worry that small amounts of antigen are eluting from your columns and, in fact, initiating a secondary immune response in your animals.

Dr. Terman: I think that the fundamental difference may be in the mode of immunization. Your animals were immunized initially intravenously and boosted intravenously. Our dogs were immunized initially in an adjuvant and boosted intravenously. But the adjuvant depot remained; and hence the chronic source of antigen was present. Radioactivity in our antigen coated columns showed no changes after our selective immunoadsorption studies, making antigen release as a cause of the observed rebound very unlikely.

I believe what you are showing us here is a reduction of antigenemia in these animals that were exchanged, and hence I think you may be reducing the antigen load, and potentially moving these animals into a state of suppression, perhaps by way of low-dose tolerance.

Dr. Branda: You mean by exchanging an animal 2 weeks after an antigen injection, there might be some soluble antigen?

Dr. Terman: Yes. BSA certainly may remain in the circulation for many months as part of an immune complex and may be metabolized very slowly. Without doing T 1/2's on your sheep erythrocytes, I would expect a sustained half-life in the circulation, as well.

Dr. Polesky: I am fascinated by your kinetics. I do want to question you about one example you used.

You showed a slide from Cimo and Aster and discussed the response in posttransfusion purpura. We had a case in Hibbing, Minnesota.... In discussing this with Dick Aster, ...he sent me some data, which I believe he has since published, pointing out that whole blood exchange was important in these cases, not plasma exchange; that the response, in terms of return from thrombocytopenia, also the bleeding and deaths, was much smaller in the group that got whole blood exchange versus just plasma exchange.

And I notice on your slide you just say "exchange transfusion." I think it ought to be clarified. Was that case a plasma exchange, or was it whole blood exchange?

And I think, in fact, Dennis Goldfinger has made the point a couple of times, that sometimes we are taking up more things than one. I think it is simple just to think about plasma exchange; and in that particular thing, from Aster's data, it appears that whole blood exchange is the important thing.

Dr. Branda: The case that I showed was whole blood exchange. But there are also, in the literature, cases treated with plasma exchange that appear to be equally effective.

Dr. Polesky: Well, Dick reviewed something like, I think, 36--34 or 36 cases. When you plot those out in terms of the return of platelet count, the bleeding problems, and deaths, the whole blood exchange does much better. I think that there is probably a difference between whole blood and plasma, because you are also removing the potential antigen in the whole blood exchange.

Dr. Branda: I wasn't aware of that data. But it is fair to say [that] in the literature there are cases that have responded to plasma exchange.

Dr. Nusbacher: I would like to develop an idea. I noticed on at least one of your slides, perhaps on the others, that the antibody level that you measured in the ITP patients did not go down-- Let me put it another way: that the platelet count went up before the antibody went away. Is that correct?

Dr. Branda: Before it goes away. Well, it depends on the assays. As you know, until very recently, there haven't been good methods for quantitating the antibody, so that even in that particular method I showed you, normal people have a measurable amount of antibody on their platelets.

So, yes; that is correct. It hadn't gone down to normal range before the platelet count went up. But as I am sure you are aware, there are a multitude of factors playing in ITP that determine platelet life span, for example, the affinity of the RE system for the antibody-coated platelets, so that the antibody level per se may not be a good correlate of what is happening. In fact, in the published studies that have looked at plasma levels of antiplatelet antibody, there isn't a terribly good correlation; and the best correlations are with antibody on the platelet surface. Unfortunately, we didn't have those techniques available at [the time].

Dr. Nusbacher: But those variables aren't applicable here, because the variable is the plasma. The status of the RE system presumably hasn't changed significantly.

Dr. Branda: But all of that, of course, affects the status of the RE.

Dr. Nusbacher: I wanted to go on a little bit, because I wanted to explore an idea. In your first patient, do you attribute the effect of the exchange to removal of the antibody or the removal of the antigen? There is at least an implication that this was thiazide-induced.

Dr. Branda: Yes. There is that implication, because I don't know what caused the ITP in that patient. But let me say that the thiazide had been administered many weeks before the exchange, and presumably that would not be a major factor, because usually in the thiazide-induced ITP's, the thrombocytopenia improves within a week or so of withdrawal of drug. So I don't think that antigen removal is a major factor, but obviously I can't prove that.

Dr. Nusbacher: Yes. I might also, to carry this a little further, like to second what Dr. Polesky said in regard to posttransfusion purpura. Antigen is in transfused blood, and it is not an autoantibody per se. Now, why the autologous platelets get removed--at least, that is my understanding--why the autologous platelets get removed is not quite clear.

Perhaps it is the adherence to an immune complex. I heard the same idea from Dr. Aster; namely, that whole blood exchange is more efficacious in posttransfusion purpura presumably because you are removing the antigen. And I would like to suggest that perhaps the reason why a patient with ITP will respond, or may respond to plasma exchange, is not because you are removing the antibody but because you are removing the antigen, which is usually unknown.

Dr. Branda: Okay. But the main thing that I wanted to illustrate with that was the effect of exchange on what was apparently a secondary immune response. And in that case there isn't any evidence of rebound. To my knowledge, there have been three or four carefully worked out kinetic studies published, in that situation.

Dr. Schmidt: We treated a case of what looked like posttransfusion purpura with what would seem to be the ideal middle component, in that we did it on the machine, but used platelet-rich plasma, and were very successful. Platelet-rich plasma is something we all used to use, but it is very hard to get unless you run your own blood center, which we do.

But it sort of solves the problem of not having to do the complete blood exchange, using the machine, and still being able to use it as an antibody sponge.

This was a case occurring about 10 days after transfusion in a classic situation. You would worry that you are putting in another dose of antigen, and perhaps immunizing again, but that did not seem to happen.

Dr. Milan Bier: Several years back, we developed a method for selective plasmapheresis whereby we remove only gamma globulins from the circulation of the blood by means of an electrophoretic membrane process. And we could remove 50 or 60 percent of the calculated circulating immunoglobulins. In short, this prevents the hyperacute rejection due to platelet antibodies when you transplant pig kidneys into dogs.

We have also done a study with this technique in sheep, which were hyperimmunized with three different antigens and invariably repeatedly removed a significant fraction of circulating antibodies to look at the rebound effect. And the rebound effect was strikingly different for the three antigens. One returned to normal, one stayed low, and one had a significant rebound. And this is where the three antigens were administered at the same time to the sheep.

Dr. Branda: Well, I think that is a perfectly valid thing. I don't think that it is worth a lot of argument about animal models for these diseases, because there is no guarantee that the animal model is going to reproduce the patient's problem.

I think that certainly in diseases like ITP, it is becoming clearer that the problem is not so much the antibody production, but the cell/cell interactions that control the antibody production. And there is no reason to believe that an ITP patient will respond as a normal dog or sheep or rabbit will. So--

Dr. Bier: Well, I wanted to say that the reaction may be highly specific and variable from patient to patient or--

Dr. Branda: Yes; I agree.

Dr. Bier: --animal to animal in antigen/antibody systems.

Dr. Branda: I agree; and that is why I don't want to make too much of the thing here.

Dr. John Egan: Dr. Branda, I don't believe that you mentioned in your cases that you treated what the replacement fluid was.

Dr. Branda: We have always replaced these with fresh frozen plasma.

Dr. Egan: To the volume that you exchanged?

Dr. Branda: Yes, sir.

Dr. Egan: Do you think that this relates to previous studies which have shown responses in ITP to plasma infusion?

Dr. Branda: Yes, we have thought about that. Those cases, to my knowledge, have been mainly in the pediatric group, and have been highly variable. I don't see how we can really exclude that possibility.

Dr. Ries: Rather than finding an answer in the kinetics of antibody response in the different patients with ITP, it may be that the patients have different kinds of antibodies, or just different titers of antibodies; and some patients will require a lot more plasmapheresis to get a response than not.

Of the two patients who didn't respond, did they get as much plasmapheresis as the ones who did respond? And if they didn't respond with 6 liters, did you try doing 12 or 15 or 20, or whatever?

Dr. Branda: The young woman who did not respond had an equal volume of a single plasma exchange. We hadn't tried repetitive exchanges; it is possible by repeating them. However, in the cases of ours that improved, and those in the literature, you generally will see some response after the first exchange. I am not aware--unlike some of the other autoimmune diseases that have been treated this way--that it is necessary to do several to see some effect. It is possible that if you treated patients that way, you may see some [change], but we haven't done that.

The young man with the Evans syndrome was severely ill and, in fact, clotted off the lines part way through the run, and we didn't restart it; so he did not get as long a run as the other patient.

Dr. Goldfinger: You are perhaps being unfair to yourself by including that last man in your statistics, the fellow with autoimmune hemolytic anemia. The thrombocytopenia and, actually, the pancytopenia seen in patients with autoimmune hemolytic anemia may be the result of the same antibody destroying all of these cells. But I think more likely it is the result of the antigen antibody reaction directed against the red cell, causing nonspecific destruction of platelets and granulocytes.

The fact that this man died from pulmonary emboli, and was obviously hypercoagulable--you mentioned he clotted off all of his lines--suggests that indeed he had autoimmune hemolytic anemia with resultant pancytopenia, and died from that complication. This was not ITP.

Dr. Branda: Yes; I think that is fair. I have tried to include it, because I have been criticized for spending more time on our successes than on our failures; so I tried to tell you about all of them, to this point.

I think, though, that from here on in, it is going to be very important for all of these patients to be reported in some detail because it is very hard for anyone to make a reasonable judgment about who should be treated this way and who shouldn't, at present....

Dr. Blumberg: It was very felicitous that Dr. Goldfinger asked that question and you came up with that answer, because I am choosing your presentation to make a statement about some of the things we have been talking about in general terms, one of which is: I am a little bit uncomfortable when I hear my colleagues making statements, based on one or two cases, that one should do things this way or not that way. I know we all have a tendency to do that, when something works spectacularly well, or spectacularly poorly.

And one of the things I perceive in the 2 or 3 years I have been involved in plasmapheresis for a variety of diseases is that the one thing we really have a lot of trouble doing is getting enough patients in any one center, or in any group of centers; and we come back to each other year after year giving anecdotal reports.

Now, I know that our center has had perhaps 20 patients over the last several years who have had...perhaps five or six different medical syndromes, ranging from myasthenia gravis to TTP to mixed cryoglobulinemia. And I know that other centers, both hospital centers and regional blood centers have equivalent experiences, which, in aggregate, clearly would, by an order of magnitude, increase the body of information available.

And I wonder if we shouldn't be spending some effort and some money, perhaps, by some agency such as the National Heart, Lung, and Blood Institute, to try to accumulate this data, so that, albeit you can't analyze it statistically in the way you would like to, we don't find ourselves in the position that the heart surgeons find themselves, 10 years into open heart surgery for coronary artery bypass, trying to prove that the 30,000 procedures they have done make some sense. And I don't expect a response to this statement on the part of any individual--I don't know how often you see cases of refractory ITP, but we might see one a year. I would like to see some progress in the field, and I think the only way we are going to get that is through the kind of hard, unpleasant, cooperative trials, whether they be randomized or not, or even retrospective, initially. That we are not doing.

Dr. Branda: Yes. I agree completely. I think there are two problems. I think one is the patient who is not only refractory but is extremely ill and dying. And in this situation it is very hard to say, "We're going to randomize you."

The second is, I think, a little easier to deal with--the patient who is refractory but stable, and not bleeding and not, obviously, going to die in the next 24 to 48 hours. I agree that any extension of this procedure to that group of patients should certainly be well controlled.

Dr. Khan: It looks like we have gone into the science of exchange philosophy; so I would like to make a comment here. ...The procedure we are doing is a very crude procedure. We are exchanging everything. If we expect very swiftly to get control results out of such a crude procedure, I think we are expecting too much.... Unless we go to some special materials like affinity chromatography, and pick single components and remove them, we will not be able to answer these questions.

In this regard, the subject of hybridoma was introduced earlier, where you make a specific antibody against a given substance; you want to remove, say, IgG. Have hybridoma against IgG and specific and pure antibody in an affinity column. Put your plasma through it, and remove one component. Then we can probably achieve some of the pure results that we are looking for. I think in another 5 years, when people look back, they will say, "Oh, in 1978-79, those people were so crude, they used to exchange whole plasma, and waste all these products."

Maybe some of the subsequent speakers will address another problem we have faced. ...when you exchange with plasma expanders, or other substitutes rather than plasma, a lot of other factors go down: trace elements and potassium. And many of these things have not been discussed. I wish some of the speakers would address some of those complications they may have run into; or were they looking for such things?

Dr. Gilcher: I think it is very dangerous to put together the retrospective data, because we are not all doing the same thing. We are not doing the same volume on a milliliter-per-kilogram plasma exchange. We are not using the same exchange media.

I think what is being pointed out here is that it behooves us to get the experts, whoever they are, together and set up some sort of prospective study; not retrospective, but prospective, so that we do the same thing, so we don't have 15 different variables. I don't think we can compare what one person is doing with what another person is doing.

Dr. Pineda: I am going to give Dr. Taft 3 to 5 minutes to present his experience with TTP. I think it is interesting because he is going to address the dose of exchange fluid we are using.

Dr. Taft: We have been very concerned with the concept of dose and frequency for plasma exchange; therefore, on all our patients with plasma exchange, we have expressed the volume of plasma exchange as the net volume removed from the patient. It is the gross volume subtracting the anticoagulant carrier.

We don't know from the literature whether a patient who is 20 kilograms or 100 kilograms, what 2 liters or 4 liters or 6 liters means in any of these patients. And we have been very concerned with this with respect to TTP, and I would just like to present our brief experience with this. May I have the first slide, please?

[Slide]

We think TTP may be a model, because we have a series of markers that most hospitals can measure fairly rapidly. We have in the panels here, the first patient we have done here, and the second patient.

The first patient is a 52-year-old woman presenting with TTP, acute renal failure, and anemia. The platelet count is shown in the open circles here, with the broken lines; serum LDH in closed circles and solid lines. High values of LDH are noted quite frequently in the literature in TTP, and we notice that because this is a high molecular weight substance, it is removed very rapidly by plasma exchange. It is virtually unaltered by dialysis.

This patient here had a simultaneous dialysis and plasma exchange the first day, and was disoriented during the procedure. The next day she was awake and alert. On the third day she had a dialysis, became disoriented again, and remained disoriented until the time the second plasma exchange was done, after which she became oriented.

We noted a very rapid reaccumulation of LDH after the first exchange, a much slower reaccumulation after the second exchange, and still slower after the third exchange. She recovered full renal function after being anuric for a total of 15 days.

The second patient was a 14-year-old boy who presented with TTP, including seizures. He was treated with various agents including 1 week of plasma transfusion, 2 to 3 units per day. He went deeper into coma. On the first plasma exchange here, [there was a] spontaneous platelet increment [to] about 6,000 from levels about 2,000. In the second plasma exchange, the platelet count went up to about 35,000 or 40,000, and then successive rises were seen in further plasma exchanges.

What we have expressed here, the numbers here, are the milliliters per kilogram of plasma exchange. All these exchanges, in both these patients, were one-for-one fresh frozen plasma.

One comment here: By using Evans blue injected into the patient before the procedure started, and measuring the Evans blue we recover, we recovered about 50 percent of the injected dose with 35 to 40 ml/kg plasma exchange. Will you go on to the next slide, please?

[Slide]

The third patient clinically had no neurologic dysfunction whatsoever until the day before she expired. In retrospect, we see a spontaneous platelet increment after only the second of five plasma exchanges; we see a much higher postapheresis value of LDH here, and we see a continuing rapid rise in the reaccumulation of LDH immediately postexchange transfusion, prior to the next day.

Clinically, she looked well. The second day a plasma exchange was omitted, and I think the sixth day a plasma exchange was omitted because her clinical condition looked very good. And she subsequently deteriorated and died within 36 hours.

Using that experience on the fourth patient, we then did plasma exchange on the night of admission of this patient. Now, where this is written in as a fraction, the top number indicates the volume in plasma exchange which is replaced as fresh frozen plasma. The bottom number here, the denominator, indicates the total volume of the plasma exchange, using essentially a plasma-free exchange.

The first night, this patient was disoriented; she became alert and oriented the next morning. We repeated the same, essentially a 4-liter exchange for this lady, again plasma-free, on the second day; and on the third day, she was disoriented again.

We increased the volume again, up to 90 ml/kg, about 6 liters for this patient, of which a significant portion was fresh frozen plasma. And it was after eight exchanges that we first began to get a definite spontaneous increment in platelet counts.

I will have to apologize for the platelet transfusions listed by the small arrows here. One of our clinicians is a little "quick on the trigger finger."

We note that the LDH, again, reaccumulates fairly rapidly until after the eighth exchange. Then we see a lesser degree of accumulation.

Now, Dr. Robert Babcock--

[Slide]

--has given us some preliminary data also, using the Dixon-Rosse technique for platelet antibody. This is measurement of serum antiplatelet antibody. The patient's serum in the acute phase reacted against the patient's platelets in the recovery phase. Now, this is, again, the first bag of collection, the last bag of collection, first bag of collection, last bag of collection here; the second patient is shown here.

And in the fourth patient, this is serum taken preexchange and postexchange, and we see rapid increments again but after essentially the eighth exchange, the antibody level stays down below the normal for this procedure. And that is when we see prolonged and sustained increments in platelet count.

[Slide]

And for the third patient, we have graphed together here simultaneously the LDH and the antiplatelet antibody. This patient, despite a much better clinical condition, had much higher levels of serum antiplatelet antibody, which we decreased by plasma exchange. But one will note that the rapid reaccumulation rate here terminally, essentially, heralded a fatal outcome.

In retrospect here, on no occasion do we reduce the LDH below 700 units. Our recommendation now is that we follow the LDH fairly frequently during the course of treatment of these patients. Essentially, we apherese first and ask questions afterwards; and finally, if we don't get the LDH down to below 400 units, [we] should enlarge the volume of the exchange.

Our data at this time do not allow any conclusion as to whether plasma replacement is necessary for the treatment of this disease, or that we are removing the antibody. We feel a little bit more comfortable about removing the antibody, knowing the antibody data here.

Dr. Pineda: Thank you, Dr. Taft....

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PLASMA EXCHANGE IN IMMUNE COMPLEX DISEASE AND GOODPASTURE'S SYNDROME

Bruce A. Pussell, M.B.B.S., F.R.A.C.P.
C. Martin Lockwood, M.B., M.R.C.P.
Hammersmith Hospital, London

Santiago R. Bartolotti, M.D.
Royal Postgraduate Medical School
London

D. Keith Peters, M.B., F.R.C.P.
Royal Postgraduate Medical School and
Hammersmith Hospital, London

INTRODUCTION

For the past 5 years we have been using a standard regimen of intensive plasma exchange (PE) and immunosuppressive therapy for the treatment of rapidly progressive nephritis, initially in the kind of nephritis mediated by antibody to glomerular basement membrane antibody (GBM/anti-GBM disease) and later in immune complex (IC) nephritis. More recently, we have attempted to assess the role of PE in less severe allergic diseases such as cutaneous vasculitis and systemic lupus erythematosus (SLE) with and without nephritis. In our assessment of the effects of treatment we have used or developed the following methods to monitor patients undergoing plasma exchange: tests for anti-GBM antibody; measurement of circulating immune complexes; assessment of complement function; and assessment of reticuloendothelial function by measurement of splenic macrophage function.

The patients studied include 28 with Goodpasture's syndrome due to anti-GBM antibody, 14 with immune complex mediated rapidly progressive nephritis (RPGN), 12 with SLE, and 2 with cutaneous vasculitis. We are attempting to evaluate the place of PE in immune complex mediated rapidly progressive nephritis by carrying out a randomized controlled trial of drug therapy plus PE versus drugs alone. That trial is in progress.

METHODS

Plasma Exchange

Four-liter exchanges were carried out daily on a Haemonetics Model 30 cell separator using as replacement fluid purified protein fraction (PPF), a solution free of detectable quantities of immunoglobulin, fibrinogen, and complement. Exchanges were, in general, carried out daily in severe disease and continued until clinical and laboratory tests showed stable improvement or until no response had occurred. The number of exchanges varied from 2 in a patient with cutaneous vasculitis to over 50 in one patient with anti-GBM antibody mediated Goodpasture's syndrome.

Drug Therapy

Patients with anti-GBM disease and IC-RPGN received a combination of prednisone (60 mg/day), cyclophosphamide (3 mg/kg/day), and azathioprine (1 mg/kg/day). In older patients (over 55 years old), the azathioprine was omitted and the cyclophosphamide reduced to 2 mg/kg/day because of the higher risk of infection. Generally, the prednisone dosage was reduced at weekly intervals and the cyclophosphamide stopped after 8 weeks or if there was any indication of bone marrow suppression.

In patients with SLE, drug therapy varied from the standard regimen described above (in those with severe nephritis) to a small maintenance dose of prednisone. In patients with SLE without severe nephritis, and in cutaneous vasculitis, drug therapy was usually stable for 2 weeks before the introduction of plasma exchange.

Anti-GBM Antibody

Collagenase-solubilized human glomerular basement membrane was prepared, and serum was tested in a solid phase assay using a radioimmune technique (4). In this assay (details of which are to be published in full elsewhere), values greater than 13 percent of a standard reference serum are always found in patients with anti-GBM disease.

Circulating Immune Complexes (CIC)

A modification (9) of the Clq binding assay described by Zubler et al. (11) was used. This assay depended on the ability of radiolabeled Clq to bind to immune complexes present in test sera. The bound Clq was then precipitated with polyethylene glycol (MW 6,000) at a final concentration of 2.5 percent. Results were expressed as micrograms bound by an equivalent amount of heat-aggregated human IgG.

Clq Metabolism

A highly purified, functionally active preparation of radiolabeled Clq was used to study metabolism in patients with SLE. Blood and 24-hour urine samples were collected and analyzed by plasma curve analysis (7) and Nosslin's integrated rate equation method (8). Values were obtained for half-life ($\tau 1/2$) and fractional catabolic rate (FCR).

Complement Function

Standard assays for complement components were carried out. In addition, complement was tested by a new method (2) which depends on the ability of complement to dissolve immune precipitates. The test can be performed in the presence of calcium and magnesium, thus testing both complement pathways, or in the absence of calcium but with magnesium, thus assaying the alternative pathway. Bovine serum albumin (BSA) and radiolabeled anti-BSA precipitates were formed and thoroughly washed. An aliquot of the radiolabeled precipitates suspended in buffer was added to a test tube and the tube counted in a gamma counter. Fifty µl of the test serum was then added and incubated at 37 °C for 60 minutes. Following centrifugation, the supernatant was discarded and the precipitate recounted. The amount solubilized was then calculated with an adjustment for spontaneous dissolution. In a group of 80 normal human sera so tested, 30 percent or more of the labeled precipitates could be solubilized (mean less 2 standard deviations).

Splenic Function

Autologous red cells were labeled with either ^{51}Cr or ^{99}Tc and either altered by coating with antibody or heat damaged (table 1) as previously described (6). Following injection, samples were withdrawn at 5-minute intervals for 30 minutes. The radioactivity per unit volume blood was measured in a gamma counter, and the half-life (τ 1/2) was determined from a semilog plot.

Table 1.--Method of damaging autologous red cells

^{51}Cr IgG-coated cells	^{99}Tc heat-damaged cells
10^6 red cells	2 ml packed red cells
50 uli ^{51}Cr (15 min)	heated 49.5 °C for 20 min
wash in saline	50 uli ^{99}Tc (5 min)
0.1 mls IgG anti-D (20 min 37 °C)	1% $SnCl_2$ (5 min)
wash twice in saline	wash twice in saline

Scanning

For quantitative scanning of the distribution of heat-damaged ^{99}Tc-labeled red cells, the patient was positioned with his back to a large field of view gamma camera so that it viewed his lungs, liver, and spleen.

RESULTS

Anti-GBM Antibody

Circulating anti-GBM antibodies were detected in all 28 patients with anti-GBM-mediated Goodpasture's syndrome. In 26 patients where renal biopsy material was available, all showed a linear distribution of IgG along the glomerular basement membrane (5).

Figure 1 shows the duration of circulating anti-GBM antibody in the 28 patients. There was considerable variation in the decline in antibody titers in those patients. Figure 2 shows a patient in whom the anti-GBM antibody fell quickly following the introduction of plasma exchange, steroids, and cyclophosphamide. Renal and lung disease were quickly controlled in the patient, and there has been no recurrence of clinical or serological disease in a period of more than 3 years.

In a group of 12 clinically similar patients presenting with oliguric renal failure, and having approximately the same titer of anti-GBM antibody, the effects of PE were analyzed. Analysis of the drug charts showed no difference in total cytotoxic or steroid drug dosage, but when the patients were grouped into those receiving 13 or more exchanges and those receiving fewer than 13 exchanges, a highly significant difference in half-disappearance time was found (figure 3).

Circulating Immune Complexes

Using the ClqB assay, CIC were not detected at presentation in any of 25 patients with anti-GBM Goodpasture's syndrome (in 3 patients the test was not performed). However, CIC were detected in 7 of 14 patients with SLE and in 1 of the 2 patients with cutaneous vasculitis. Figure 4 shows serial studies in a patient with SLE who presented with septicemia complicating undiagnosed SLE. Following initial therapy with antibiotics and corticosteroids there was a decrease in CIC and improvement in renal function. Further therapy with azathioprine and then plasma exchange led to a further diminution in CIC and improvement in the ability of the patient's serum to solubilize *in vitro* IC.

Figure 1.--The duration of anti-GBM antibody in 28 patients with anti-GBM disease. There is considerable variation in the duration of antibody titers in these patients.

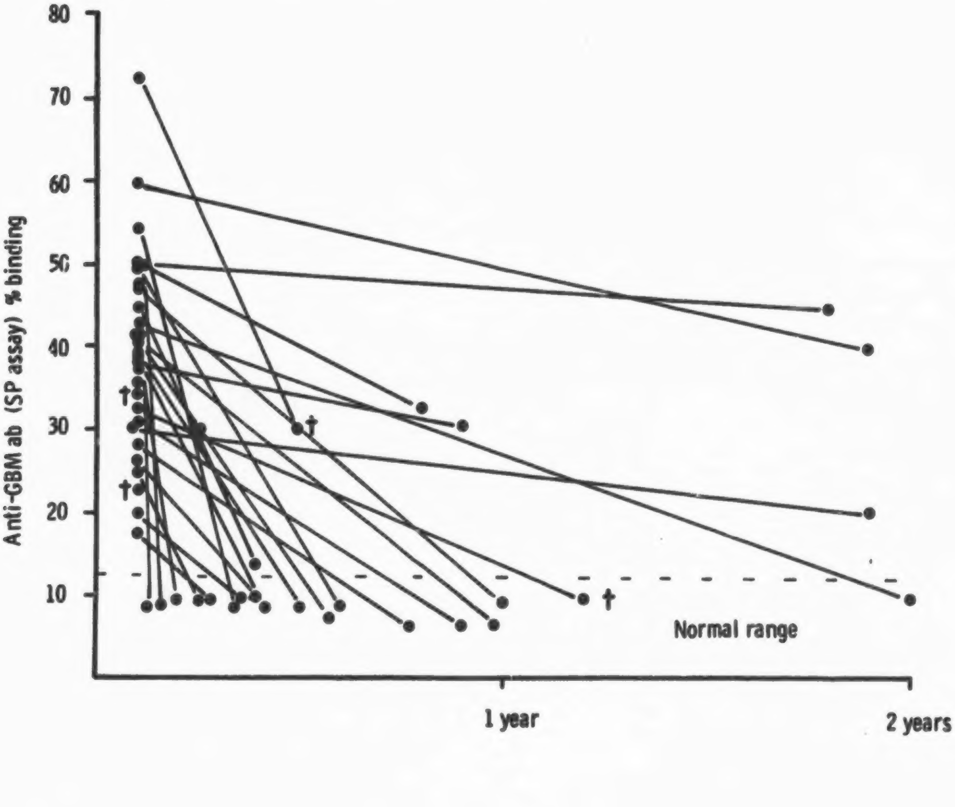


Figure 2.--A patient with anti-GBM disease who had a rapid response to plasma exchange and drug therapy.

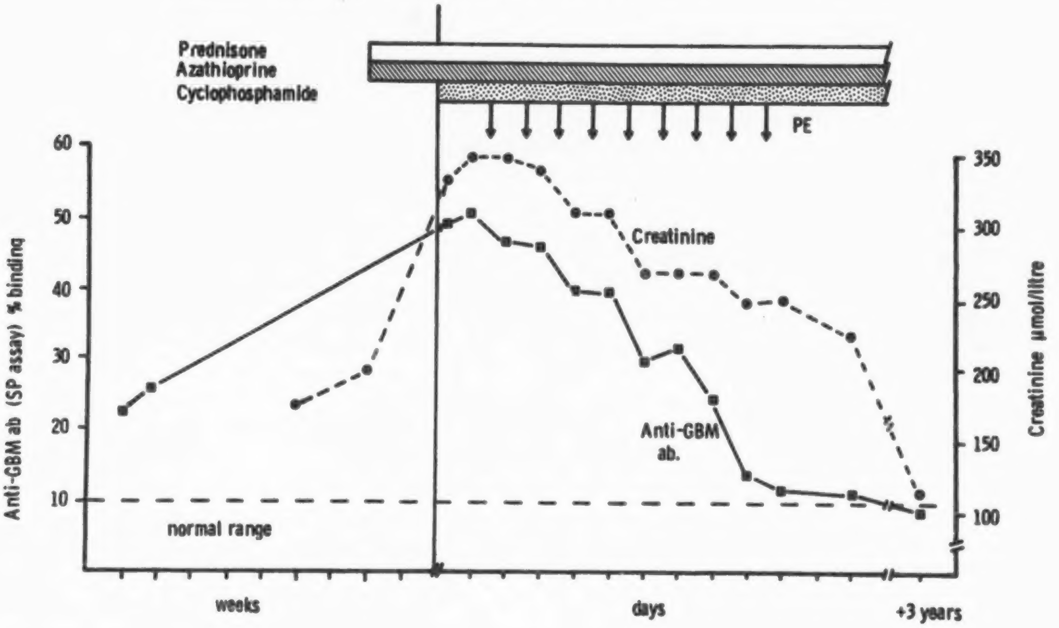


Figure 3.--Note that in patients with similar antibody titer and drug therapy, the group who received 13 or more plasma exchanges had a more rapid disappearance of anti-GBM antibody.

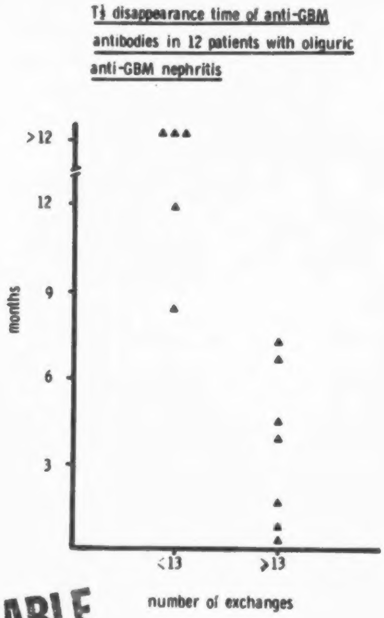
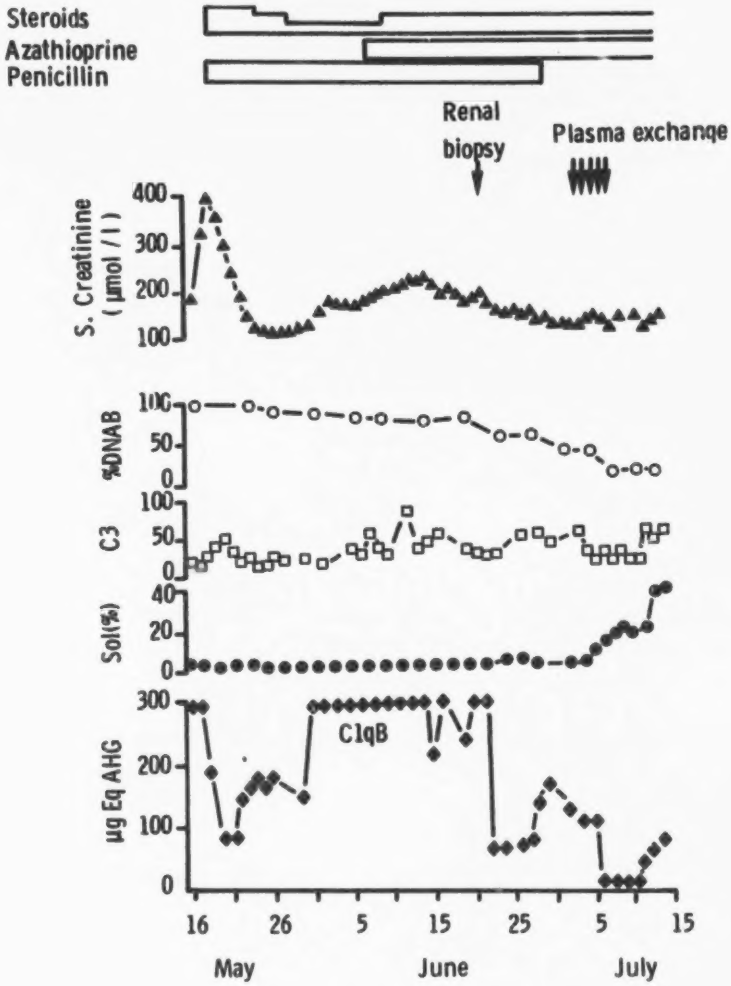
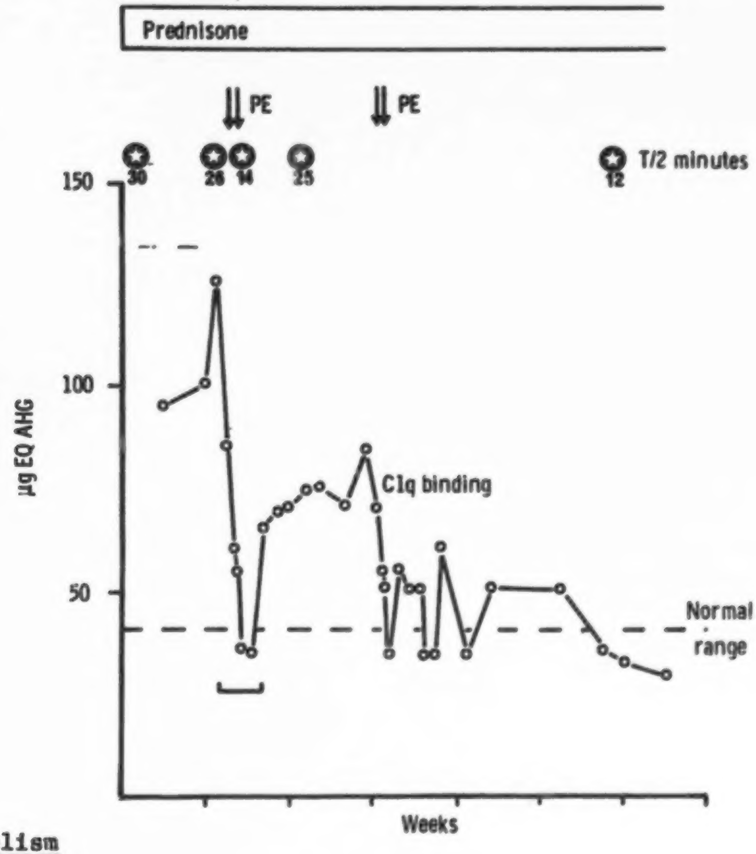


Figure 4.--A patient with septicemia and SLE. Note 1: The relationship between ClqB and renal function in the early part of the graph. 2: The very low values for the solubilization assay which improve following the period of plasma exchange. Sol = Solubilization assay (%).



An interesting and common finding in patients with IC disease treated by PE has been a continuing fall of the CIC beyond the end of the plasma exchange period. This is illustrated in figure 5.

Figure 5.--Results of the ClqB assay (open circle) and τ 1/2 in minutes (stars) for heat-damaged red cells in a patient with SLE treated with plasma exchange. Note the close inverse relationship between ClqB and τ 1/2 red cell clearance and the continuing fall of ClqB beyond the end of plasma exchange.



Clq Metabolism

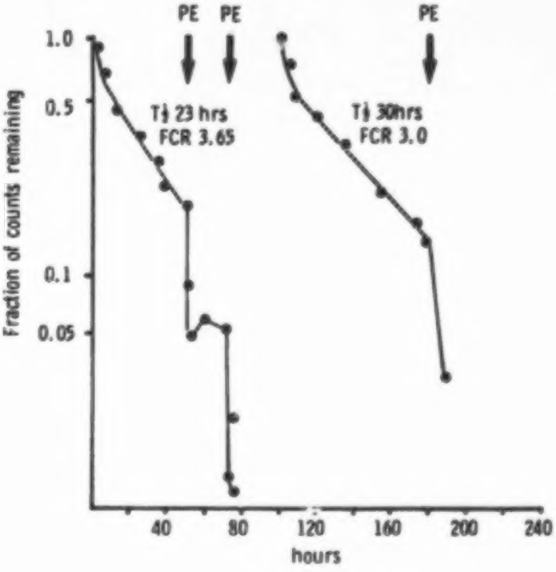
Table 2 gives the results of half-life (τ 1/2), fractional catabolic rate, extravascular distribution (EV/IV ratio), and extravascular catabolic ratio from Nosslin's analysis. In the patients with SLE, not only was the half-life faster and the FCR higher, but the Clq had an increased extravascular distribution and catabolism. Figure 6 illustrates a patient with SLE studied before and after plasma exchange with no other alteration in therapy. Both the τ1/2 and FCR were altered toward normal following two daily, 4-liter plasma exchanges.

Table 2.--Clq metabolism

	Number	τ 1/2 hours	FCR %/hr	EV/IV Ratio	EV Cat Ratio
Normal	8	37	2.85	0.50	0.97
SLE	6	24*	4.40	1.36*	0.47*
RA	9	33	3.60	0.48	1.47
+ve ClqB	15	29*	4.00*	0.81	1.05
-ve ClqB	10	32	3.20	0.97	0.59

τ 1/2: half-life
FCR: Fractional catabolic rate
EV/IV ratio: extravascular-intravascular ratio
EV cat ratio: extravascular catabolic ratio approximates unity if EV catabolism is negligible, and values less than one indicate the degree of EV catabolism. Note, there is increased τ 1/2, FCR, EV/IV ratio, and EV catabolism in SLE compared to normals.
* P <0.05

Figure 6.--Plasma disappearance curves of radiolabeled Clq given to a patient with SLE before and after plasma exchange with no other alteration in therapy. Note that the τ 1/2 and FCR have been altered toward normal following two 4-liter plasma exchanges.



PE - plasma exchange

Complement Function

Defects in the complement-dependent solubilization assay were found in six of seven patients with SLE and both patients with cutaneous vasculitis. Although only a few patients were tested, no abnormality was found in those with anti-GBM disease (four tested) or IC-mediated RPGN (four tested).

An example of the changes in the complement-dependent solubilization assay is shown in figure 4, which describes a patient with SLE and septicemia referred to previously. The ability of the patient's serum to solubilize immune precipitates remained at or near zero, despite improvement in other variables, until she was given a course of five daily plasma exchanges. Despite the replacement fluid being PPF (i.e., it contained no complement components), there was a striking improvement in test results following plasma exchanges.

Splenic Function

Damaged autologous red cell clearance was performed on two patients with anti-GBM disease, two patients with IC-RPGN, five patients with SLE, and two patients with cutaneous vasculitis. Details have been reported elsewhere (6). Figure 7 illustrates the half-life ($\tau 1/2$) of IgG-coated red cells in a patient with cutaneous vasculitis. Following plasma exchange, the $\tau 1/2$ of heat-damaged red cells in a patient with SLE paralleled the level of CIC. Following two periods of plasma exchange both CIC and splenic function improved. Figure 8 shows the quantitative scan of a patient with IC-mediated RPGN before and after plasma exchange. The abnormal distribution of the damaged red cells in the lungs and liver has been altered by plasma exchange, and the splenic uptake has been improved.

DISCUSSION

In anti-GBM antibody-mediated Goodpasture's syndrome, the level of anti-GBM antibody in individual patients studied serially generally showed a good correlation with disease. However, plasma exchange results in depletion of globulins as well as specific antibody and, therefore, removes inflammatory mediators such as complement and clotting factors; this could contribute to the beneficial effect of the procedure. In that connection, we have observed (10) that intercurrent infection can exacerbate anti-GBM disease without any significant change in circulating antibody concentration. We have postulated that this is a mediator effect of the acute phase response. It is necessary to consider the relative contribution of PE and drug therapy to the reduction in circulating antibody level. In an attempt to

Figure 7.--IgG-coated red cell clearance. Following two 4-liter plasma exchanges there is sustained improvement in the τ 1/2. Shaded area indicates the normal range.

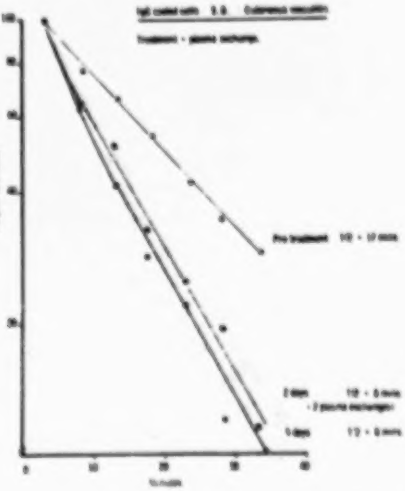
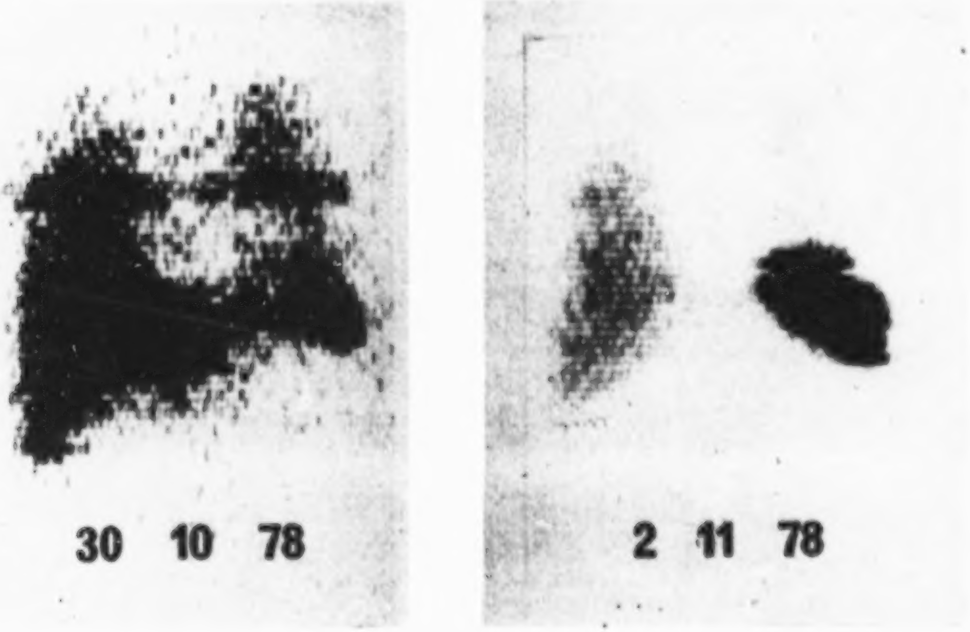


Figure 8.--Quantitative scan in a patient with IC nephritis. The abnormal distribution of altered red cells, with uptake to the lungs, liver, and spleen, seen on the left has been altered by plasma exchange to a more normal pattern with predominantly splenic uptake, on the right.



determine what that contribution is, we have analyzed the time taken for the antibody level to fall in a group of patients with similar degrees of renal impairment, antibody level, and drug therapy. In the group, the disappearance of antibody depended on the amount of plasma exchanged. Those patients who received more intensive plasma exchange had a more rapid fall in antibody concentration. Although various explanations may exist, of particular interest are the results of the experiments of Hall et al. (3), which led to the concept of autoimmune amplification. Following infusion of IgG subclass-specific antitubular basement membrane (TBM) antibody into susceptible guinea pigs it was shown that they developed an IgG subclass-nonspecific auto-anti-TBM antibody which contributed to the renal damage. It is therefore (as postulated by Hall et al.) possible that PE may interrupt such a mechanism. However, other possibilities ought to be considered, including the removal of GBM/anti-GBM complexes from the circulation.

In patients with IC disease treated by plasma exchange, there was a convincing relationship between the level of CIC, the response to PE, and disease activity. Plasma exchange in that group of patients was aimed at reducing the load of CIC. We were particularly interested in the question of whether testing for CIC identified a group of patients likely to respond to treatment by PE. That did appear to be the case with RPGN: of nine patients with a good response to PE therapy, seven had CIC which fell to undetectable amounts following therapy; CIC were frequently detectable at high concentrations in the group of patients with SLE, reflecting the severity of the disease in those selected patients. An observation of special interest in three of our patients was the continued fall in CIC in the period immediately following plasma exchange. This, along with the demonstration of reversible splenic blockade in our patients, provides evidence that reticuloendothelial clearance by plasma exchange allowed the continued fall of immune complexes to occur.

Using damaged autologous red cell clearance as a test of splenic function, we have been able to show that splenic blockade is a common feature of patients with putative immune complex disease. Furthermore, the splenic blockade is reversible by plasma exchange and bears an inverse relationship to the level of CIC in those patients in whom the ClqB assay has been positive. It is interesting that reversible hyposplenism could also be found in patients without detectable circulating immune complexes. It is possible that our test for CIC was insensitive in those patients and that more than one assay should be used in monitoring patients with immune complex disease. If defective splenic clearance is representative of defective reticuloendothelial function as a whole, the finding of hyposplenism in our patients raises the possibility that continued disease activity may not be caused by the original antigen-antibody complex. Once splenic blockade has occurred there would then be delayed clearance of any immune complex formed in response to other environmental antigens. This is a possible explanation for the difficulty so far experienced in isolating specific antigens from immune complexes.

We have reported previously (9) the value of conventional tests of complement in patients with immune complex disease. By studying *in vivo* metabolisms of Clq in our patients, we have been able to show a strong association between hypercatabolism and the presence of CIC. Such *in vivo* study provides evidence that material detectable in an immune complex assay is capable of causing complement activation in patients and may therefore have phlogistic potential. That dissolution of tissue-bound immune complexes is a function of complement has been suggested by the experiments of Bartolotti and Peters (1) in the experimental model of acute serum sickness in rabbits. Decomplemented animals had delayed removal of renal-bound immune complexes. We have therefore applied a new test of complement function (the solubilization assay) because it is able to test complement in one go as well as being able to detect anti-complementary substances (such as immune complexes). In many of our patients the solubilization test was the last to return to normal following therapy. On the basis of our findings, this sensitive but simple assay may prove to be a good and clinically important test for complement function.

In conclusion, we believe that the use of a variety of methods of monitoring patients undergoing plasma exchange has led to a greater understanding of the complicated pathogenic mechanisms. The role of plasma exchange in immune complex disease awaits the results of controlled clinical trials.

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DISCUSSION

Dr. Terman: I think that solubilization assay is very interesting. How does that correlate with the C3 levels? And is the capacity to disrupt precipitates restored if you employ...fresh frozen plasma as replacement material, rather than PPF?

Dr. Pussell: To answer the question in a couple of paths--firstly, there is a direct correlation between the level of C3 and the ability to solubilize, but that is not the whole story. The solubilization, the ability of serum to solubilize immune precipitates, is absolutely alternative-pathway dependent; but it can be augmented by about 20 to 25 percent by classical pathway assistance, also. So that it is an overall test of complement function, we think.

We have looked at patients with infusions of fresh frozen plasma rather than plasma exchange. In actual fact, the plasma that you infuse is capable of solubilizing immune precipitates. So it is not surprising that after an infusion of plasma, there is an improvement in the solubilization performance, by the patient's sera. But it is generally not as prolonged as this patient we have seen here. Following a plasma exchange as the only new form of therapy, this solubilization assay improved dramatically, and was sustained.

Voice: What are you using to exchange?

Dr. Pussell: Albumin....

Dr. P. A. Gordon: On your 28 patients with Goodpasture's, could you comment on the status of renal function in this group, let's say, 6 months after discharge? And second, could you comment on the rather disappointing results reported recently by the group in Edinburgh?

Dr. Pussell: Yes. The group in--Well, in our patients, I can't recall the exact figure, but it has all been published, as you are probably aware.

The renal function in all but one patient has been preserved if they have had renal function at time of presentation. Now, I don't wish to imply that the patients who don't have any renal function at presentation should not be plasma-exchanged; because I think the life-threatening pulmonary hemorrhage in such patients is well-controlled by plasma exchange.

The Edinburgh group--their experience is far less than ours. I think that paper was on two patients only. We would disagree with it, entirely.... In fact, we have talked about controlled trials here. We would...not in any way anticipate, or even consider, instituting a controlled trial for Goodpasture's syndrome mediated by antiglomerular [basement membrane antibodies]. We don't believe that renal function, or the patient's life, should be risked by such trials....

Dr. Grossman: I have got a comment and a question. The comment is that, getting involved with this and listening to people talk and reading the literature is sort of like sitting and watching my 2-year-old play with his little shape toy. ...It is a toy that has three or four little holes of different shape in a box, and the child has a set of blocks that are supposed to fit into the representative holes.

And what we have is a whole host of diseases that we are all trying to fit into one hole; and unfortunately, the blocks that we are using obviously don't fit. And I think the "hole" basically is our concept that these immune diseases are mediated by the antibodies, or possibly by the immune complexes, that we are measuring in the circulation.

And I think that clearly, from the comments that have been coming out here, is a very naive interpretation of the data. We need to know more about the individual antigens, as somebody has already pointed out, and their antigenicity. We need to know more about the antibodies, the kinetics of the antibody, the interactions between these substances, the interactions between the antigen/antibody complexes and their target organs, and we need to know more about the RE system. And I think until we get that sort of knowledge, we really don't have any alternative but to forge straight ahead.

But I would emphasize that in all these patients that are being treated and being reported, as much knowledge [as is possible] should be derived from each individual patient...

I think it is equally naive, as people have commented, that there may be technical differences that are resulting in the discrepancy between data from different centers. And I think it is more likely that because these diseases are so heterogeneous, that it is not the techniques that are so much different, but the diseases that we are treating, labeled as the same, but in fact...very different.

And the question I really have, as a nonimmunologist--as a hematologist but as a nonimmunologist--is how strongly people here feel that, for example, a single antibody is the cause celebre for any particular disease?

For example, the group from Hammersmith has clearly shown that anti-GBM assays do not correlate with clinical activity of disease. They do not correlate from patient to patient; and they often don't correlate within an individual patient. And I wonder how much time we are wasting on developing assays for specific antibodies when this may be only a very small fraction of the problem.

Dr. Pussell: Well, the first comment that I have is that I must disagree. I think that anti-GBM disease is caused by anti-GBM antibody. That we are unable to completely explain the association between deterioration and disease activity, or deterioration and end organ function, without correlating that to a change in antibody level, just explains that we don't understand the whole process; but that anti-GBM disease is caused by the circulating antibody is shown very well by the fact that if you transplant these patients when they still have circulating antibody level, they get recurrence in the graft 100 percent of the time.

If you do not transplant them until that antibody level has fallen to zero, they do not get recurrence in the graft; so I think that in this particular disease process, the antibody is the cause of the disease.

...You talk about naivete. It is also naive to think there is any one immunological event occurring in a patient, and it is quite possible that you may have two. There is no reason why you cannot have one type of hypersensitivity reaction, such as immune complex disease, being superimposed upon an antibody-mediated disease.

If you have a damaged organ and an immune complex disease secondary to an antibody disease, there is no reason why that organ might not be further damaged by deposition of immune complexes by complement activation, and the consequences of that. I think what we are looking at is a combination of events, in such patients.

Dr. Polesky: I'd like to ask a practical question. I think your theoretical studies and your measures are very nice, but you talk about PPF and then you talk about albumin. They are two different things. Plasma protein fraction, at least in this country, is not the same as 5 percent albumin. And the reason I raise the issue and the question is that the package insert for PPF has been recently changed to point out that rapid administration is associated with hypotensive effect.

And if you are using plasma protein fraction...in these procedures, the rates of flow are pretty high, and you return pretty fast. Have you seen hypotension as a consequence, in the recipient, perhaps from the large and rapid reinfusion of PPF?

Dr. Pussell: Yes. There have been reports from Australia, as well as the United States, about hypotensive effects of this exact same preparation. It does not occur [in], and there have been no reports of, the use of the preparation prepared by the Lister Institute in the United Kingdom. It is well known that PPF prepared both in Australia and here can cause hypotension. The preparation of the property of the protein in the United Kingdom has not been reported to cause hypotension. There must be some slight variation in the method of preparation.

Dr. Cuttner: I would like to make a general comment. There have been many statements about randomized trials--including the present speaker, who gave a very good talk, saying that he could not possibly participate in one.

As somebody who participates in loads of randomized trials with many patients who are, if not about to die tomorrow, certainly critically ill, I would say the main problem one has is if you don't have a bias that one treatment is better than the other, it is very easy to randomize patients.

My other comment is that most of the studies that have been reported today, which are very interesting, in fact are case reports. And the only way that we are ever going to get any real knowledge is if we can gather a large number of patients that are treated in one or two ways, and if everybody will agree to do it, and that is the only way we will ever accumulate a large body of knowledge.

Dr. William Clark: I just wanted to ask you what your complement kinetics, your Clq-- There was no significant difference, I assume, between your groups. Is that correct?

Dr. Pussell: There was a significant difference between the normals and the lupus group, and the normals and the positive Clq-binding group. There was no significant difference between the normals and the negative Clq-binding group, or the rheumatoid arthritis group, although there are only two patients in the rheumatoid arthritis group.

Dr. Clark: So you had just an excellent recovery and a very little standard deviation, I would gather?

Dr. Pussell: We had a small standard deviation, yes, sir.

Dr. Bier: From my limited past experience with renal allograft, trying to modify renal graft response, the antibodies were the recognition factor, but the complement was the destructive factor. And one could obtain significant prolongation of renal grafts by either selectively absorbing or removing the antibodies, or by inactivating complement.

Now, ...in your group of diseases that you discussed, is there any disease that could be helped or alleviated, temporarily, by abolishing complement, rather than by removing antibodies, or immune complexes?

Dr. Pussell: In the group of patients with anti-GBM nephritis, we have found no complement abnormalities, in measuring complement paths, or in looking for complement breakdown products. And indeed, in experimental animals, the proteinuria cannot be relieved by decomplementing the animals.

Dr. Bier: I see. Now, second question: Would any of these treatments be of use in acute rejections...of transplanted kidneys?

Dr. Pussell: Well, people have been using plasma exchange in the treatment of transplant rejections.

Dr. Bier: And how effective is it?

Dr. Pussell: It's variable. The cases are much varied as with ITP--

Dr. Bier: Wouldn't this constitute the biggest number of patients that could be randomized, because there are probably more of those patients than any other group of immune disorder patients that could be randomized, possibly?

Dr. Pussell: I am afraid that most transplant physicians would consider steroids much more effective in treating transplant rejection than plasma exchange; and you may have trouble in participation, in such a trial.

Dr. Bier: I would like to add, then, a final statement rather than a question, if I may. There was a great deal of discussion, and tomorrow there will be more, about selectivity of response; and the people have mainly been looking for selectivity by means of affinity chromatography.

I would like to say that there are other means of selective fractionation, and we have procedures which are based on three different principles: One is on electrical charge of the proteins; the second is on the solubility of the proteins; and the third is on sensitivity to oxidizing agents.

We have shown several years back that the complement can be very effectively inactivated, rather than activated, by selective oxidation. Thank you.

Dr. Pussell: Thank you.

Dr. Joshua Levy: I think it is obviously imperative that we address the question: Why do we have such variability between different investigators dealing with the same disease? And why do we have, in fact, variability... with a single investigator, between different patients? And does that give us some clues as to whether or not we really can not use a single standard approach, but would have to tailor our approach in plasmapheresis for the individual patient?

Earlier this morning, we heard about cytapheresis, and I think all of us were willing to accept quite well the difference in proliferative rates of leukocytes among different leukemia patients as explaining why we can adequately deplete some, and not others. I think if we use a similar concept with these antibodies, perhaps we can very well appreciate that these patients are making antibodies at different rates.

A number of years ago we published in the JCI a paper on the rate of immunoglobulin synthesis in SLE and in rheumatoid arthritis. And we were surprised to find that despite perhaps normal serum levels of immunoglobulins, some lupus patients were making four times as much immunoglobulin, Ig, as normals do. And this...could not be appreciated by looking at their serum Ig levels.

So the rate of formation of immunoglobulin, and these specific antibodies, certainly may be the limiting factor and our ability to remove those antibodies is going to be a key feature. The question that would raise, of course, is: If you start apheresis on a patient and find that, in fact, his antibody level is not decreasing, would you increase the frequency of apheresis and the volume of apheresis?

Dr. Pussell: Well, all other things being equal, the half-life of normal IgG is 21 days, or something like that. And the half-life is determined on the antibody specificity; in other words, whether there is antigen around to mop it up, primarily.

Dr. Levy: It could be a combination of all those things.

Dr. Pussell: Yes. All those things; so that if we perform daily 4-liter plasma exchanges, and that is approximately one blood volume, with the reservations described before...the antibody level doesn't fall, from day to day, and level off.

Dr. Levy: I think the question would be, probably more appropriately, the frequency. It may well be that if we can reach a certain level of removal of antibodies or immune complexes, that the feedback mechanism which may be maintaining that immunization will be curtailed.

I would like to get some other opinions, as well, as to whether or not the Lockwood group, perhaps, is more aggressive than most other centers, in terms of the volume and the frequency of their plasmapheresis?

Dr. Pussell: Well, that's rather strange. Just one other thing: It cannot be that in anti-GBM disease, the antigen turns off production, because even in patients with renal function, the antigen remains. And this antibody does cross-react with lung, as well. It is antibody synthesis which is turned off on this particular group of patients. And I don't know whether one should really evoke the network theory or whatever other controlling mechanisms there are for antibody production, but perhaps this is being influenced.

Dr. Goldfinger: Your group has had great success using the combination of plasmapheresis and immunosuppression. Would you consider eliminating the immunosuppression, based upon some of the things we have discussed today concerning--perhaps its lack of an important role in slowing down antibody production in the situation; and secondly, the theoretic basis for the inclusion of this material: Was it included because it is known that it will prevent antibody rebound? Does this have to be modified based upon new knowledge that perhaps it doesn't modify antibody rebound? Or was it included because it was just there before? So often we have seen steroids used this way, because they have been used to treat a disease and really, they have been totally ineffective in treating the disease, but everyone who comes along with a new treatment is afraid, for some reason, to drop the steroids, perhaps because of one good report.

My understanding of this disease--and I must say we have had good success using your approach, in some patients--but my understanding has been that immunosuppressive therapy has been ineffective in treating this disease. Why is it continued?

Dr. Pussell: Initially,...in 1973, when this was started, the immunosuppressive therapy was added to suppress the long-term production of antibody, and the plasma exchange therapy was instituted to suppress or eliminate the current circulating antibody. We wouldn't dare change it. It works too well.

Now, why it works, is-- Perhaps we can discuss this too. Maybe it is not plasma exchange itself, in removing the antibody, that leads to its working. Maybe we are altering the effectiveness or distribution of the drugs we're giving. And there is some evidence to this; and I hate to admit it, but there is a group in Australia who are treating hypertension with plasma exchange. And in this particular situation, maybe they really are altering effectiveness or distribution of the drug therapy by altering the protein binding capacity.

It is something to think about. We wouldn't alter the therapy at this stage. The only qualifications I gave you were that in elderly patients, thiazophiaprine is dropped, and we are a bit more cautious with the cyclophosphamide. The cyclophosphamide is stopped after 6 weeks, usually, in the anti-GBM patients.

In the other patients, it is continued. For instance...in rapidly progressive nephritis [it] is continued for longer than that.

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PLASMAPHERESIS IN NEUROLOGIC DISORDERS*

Peter C. Dau
Paul M. Aggeler Memorial Laboratory
Children's Hospital of San Francisco
Department of Medicine
University of California
San Francisco, California

Jon M. Lindstrom
The Salk Institute
San Diego, California

Eric H. Denys
Paul M. Aggeler Memorial Laboratory
Children's Hospital of San Francisco
San Francisco, California

This report is an assessment of the results in carrying out 882 plasmaphereses in 55 patients with neurologic disorders, including 32 with myasthenia gravis (MG), 14 with idiopathic inflammatory myopathy (IM), 8 with multiple sclerosis (MS), and 1 with the myasthenic syndrome (table 1). Treatments were usually carried out weekly or less often if prolonged therapy was required. Each plasmapheresis was done on a continuous-flow basis with an Aminco cell separator, and an amount of plasma equivalent to one plasma volume calculated as 4 to 6 percent of the body weight was exchanged each time. Plasma substitutes consisted of a combination of lactated Ringer's solution, dextran-75 in normal saline, and purified protein fraction, all containing physiologic concentrations of Mg^{++}, Ca^{++}, and K^{+} (table 2) (1).

MYASTHENIA GRAVIS

Eighty-seven percent of patients with myasthenia gravis have an autoantibody which reacts with the nicotinic acetylcholine receptor (AChR) (2). Its specificity gives it an appropriate binding location which could account for the disturbance in neuromuscular transmission found in MG, and changes in anti-AChR antibody (ab) concentration have

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Table 1.--Neurologic diseases treated with plasmapheresis

Disease	Number of Patients	Number of Plasmaphereses
Myasthenia gravis	32	526
Idiopathic inflammatory myopathy	14	197
Multiple sclerosis	8	144
Myasthenic syndrome	1	9
Totals	55	876

Table 2.--Technical aspects of plasmapheresis

Access to circulation:	antecubital vein, femoral hemodialysis catheter, femoral arteriovenous shunt
Amount exchanged:	4% to 6% of body weight (3 to 5 liters)
Duration of exchange:	2 to 5 hours
Frequency of exchanges:	weekly, as tolerated
Replacement fluids:*	
1. Dextran-75 in saline	(0.5 to 1.5 liters)
2. Purified protein fraction	(1.0 to 2.5 liters)
3. Ringer's lactate or saline	(0.5 to 2.0 liters)

* Contain physiological concentrations of Mg^{++}, Ca^{++}, and K^{+}.

been found to correlate with changes in clinical parameters (3-10). The content of AChR is reduced in MG muscle and half of the remaining AChR has bound immunoglobulin (11). Our 32 MG patients either had had an inadequate response to conventional therapy (anticholinesterase drugs, corticosteroids, thymectomy), or conventional therapy had been deemed contraindicated or inappropriate (due to preexisting diabetes mellitus or hypertension in the case of corticosteroids, advanced patient age or long-standing disease in the case of thymectomy). Thus, 24 out of 32 had had thymectomy (12 thymomas), and 26 out of 32 had been treated with corticosteroids. All had generalized disease of at least moderate severity (Modified Osserman Classification: 11 II B, 7 III, 14 IV), and 13 were entirely or partially respirator-dependent.

The MG patients were treated with an initial series of plasmaphereses at weekly intervals, except for five early patients treated more frequently because Scribner arteriovenous shunts were present. The endpoint of a series of plasmaphereses was determined clinically, based on three parameters: complete or near remission; lack of further clinical improvement (especially in chronic MG); and absence of a tendency to develop recurrent symptoms in the few days prior to the next plasmapheresis. Azathioprine, 2.5 to 3.0 mg/kg body weight, was begun on the day of the first plasmapheresis. The dose of corticosteroids used was quite variable, limited by each patient's previous experience with side effects; the patients usually came to us taking the maximum dose of corticosteroids that they had been able to tolerate. In such cases, the preexisting corticosteroid therapy was simply continued; in others, an attempt was made to increase it to 60 to 80 mg of prednisone daily during the initial plasmaphereses, and in all cases an attempt was made to taper the administration of prednisone so that it was given only on alternate days (25 to 100 mg) once substantial clinical improvement set in. Four patients were treated with plasmapheresis and azathioprine, without corticosteroids. Following the phase of plasmapheresis, the patients were maintained on immunosuppressive and anticholinesterase medications alone.

The results of plasmapheresis treatment in patients with MG are given in tables 3 and 4. Muscle strength, as determined by vital capacity and arm extension time, significantly increased while the requirement for anticholinesterase medication fell. The EMG decremental response lessened significantly. Thirteen patients were entirely or partially respirator-dependent; all of them became independent of the respirator during their initial series of plasmaphereses, and only one of them relapsed, because therapy had to be withdrawn (vide infra). The clinical response was striking in many of the 19 patients achieving stable improvement--three of them had complete clinical remissions--although some achieved only moderate improvement. Only three were not treated with our standard regimen of both prednisone and azathioprine during and after plasmapheresis (3): one was treated with azathioprine alone, one with cyclophosphamide alone, and one with a combination of

Table 3.--Parameters of myasthenia gravis severity before and after a series of plasmathereses

Parameter	Number of Patients	Before	After	p**
Vital capacity (liters)	32	1.66±0.13*	2.75±0.15	< 0.001
Arm-extended time (sec)	32	38±8	203±24	< 0.001
Daily pyridostigmine intake (mg)	32	690±7	329±6	< 0.001
EMG decremental response (deltoid)	22	41±6	24±4	< 0.01

* ± S.E.M.

**† Paired t-test

Table 4.--Response to plasmapheresis in 32 myasthenia gravis patients

Response	Number of Patients	Mean Number of Treatments	Mean Followup*
Stable improvement	19	11	9
Partial relapse	7	13	7
Maintenance	4	48	
Complete relapse	1	28	12
No response	1	10	9

* Months since last plasmapheresis.

prednisone, cyclophosphamide, and vincristine. In each case, improvement was relatively stable upon cessation of the series of plasmartheresses and during an average followup period of 9 months. Ten have been able to tolerate a reduction in dosage of immunosuppressive medication. Some of these patients showed a mild deterioration or continued to gradually improve following cessation of plasmapheresis, but in all cases the bulk of their clinical improvement occurred during the phase of plasmapheresis when a mean of 11 treatments were carried out. These clinical impressions are corroborated by the changes in anti-AChR ab titers, measured just before each plasmapheresis and during followup in 14 stable MG patients in whom followup titers were available, and who were treated continuously with prednisone and azathioprine both during the phase of plasmapheresis and the followup period. Thus, the mean decline in anti-AChR ab titer (figure 1) registered during the phase of plasmapheresis at 4 weeks was 65 percent; at 7 weeks, 68 percent; and at 11 weeks, 72 percent. Followup titers at 5 and 9 months still showed a mean decline of 65 and 69 percent, respectively. These data strongly suggest that the great clinical improvement seen in our patients during the phase of plasmapheresis was due to the rapid lowering of anti-AChR ab titers produced by the combined action of plasmapheresis and immunosuppressive drug therapy. Single plasmaphereses carried out without immunosuppression do not produce sustained clinical improvement or reduction in anti-AChR ab titers (6); and the effects of immunosuppression alone on anti-AChR ab titers and MG symptoms are more gradual and of less magnitude than when combined with plasmapheresis (5,6,8-10). Subsequent treatment with immunosuppressive drugs alone did not produce a further decline in anti-AChR ab titers and provided only modest, if any, further clinical improvement.

The changes in anti-AChR ab titer occurring during plasmapheresis and subsequent followup are illustrated in figure 2. The patient, a 68-year-old female, was doing poorly despite surgical removal of her benign thymoma 1 year previously and subsequent treatment with prednisone, 75 mg q.o.d. Prior to plasmapheresis she was taking 780 mg of pyridostigmine daily for moderate generalized weakness. Her arm-horizontal time was 13 seconds, and she could only manage a few steps with the aid of a cane. She manifested gradual improvement during a course of nine 3-liter plasmaphereses (body weight 76 kg). Azathioprine, 175 mg daily, was begun at the time of the first plasmapheresis. Her strength gradually improved so that by the end of her series of treatments she was able to reduce her pyridostigmine dose to 270 mg daily, her arm-horizontal time was 68 seconds, and weakness was not demonstrable on muscle testing. The anti-AChR ab titer had been reduced to 28 percent of the preplasmapheresis value. She was maintained on prednisone and azathioprine; 6 months later the anti-AChR ab titer was 29 percent of the original titer. At followup 1 year after initiation of plasmapheresis, her pyridostigmine requirement had dropped to 90 mg daily, the arm-horizontal time was 150 seconds, and she reported increased stamina. Her anti-AChR ab titer had fallen to 21 percent of

Figure 1.--Serial determinations of antibody to acetylcholine receptor (anti-AChR). Percentage of the initial titers in 14 myasthenia gravis patients attaining stable improvement through plasmapheresis combined with prednisone and azathioprine therapy.

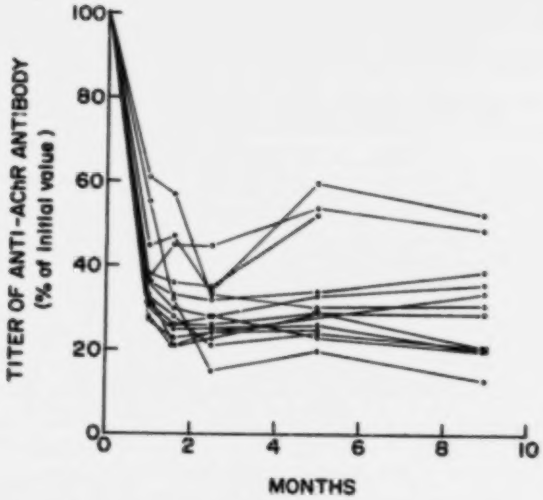
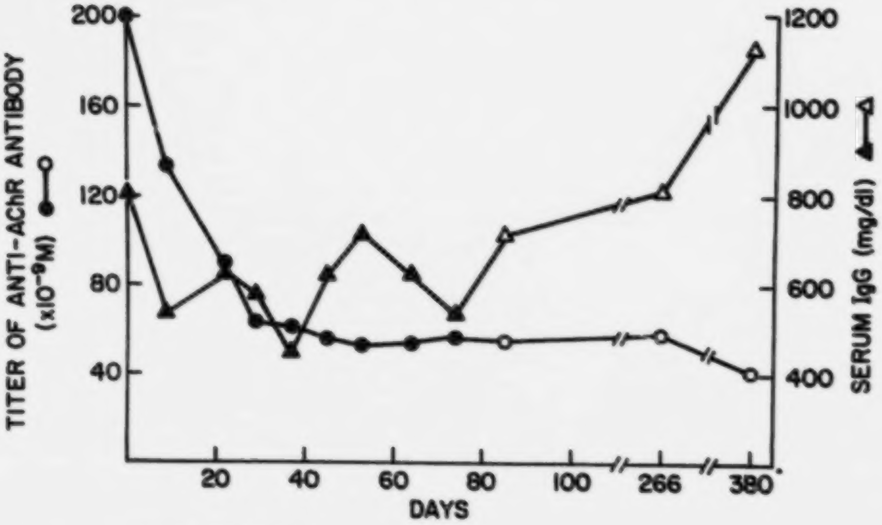


Figure 2.--Serial determination of titer of antibody to acetylcholine receptor (anti-AChR) and serum IgG in a myasthenia gravis patient. The antibody was measured at the beginning of each plasmapheresis (closed circles) or during the followup period without plasmapheresis (open circles). IgG was measured at the beginning of each plasmapheresis (closed triangles) or during the followup period without plasmapheresis (open triangles).



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the original titer. Therefore, under conditions of constant immunosuppressive drug therapy, the patient's anti-AChR ab titer fell by 72 percent during the 3-month phase of plasmapheresis, but only a further 7 percent during the 9-month followup period. The bulk of the patient's clinical improvement and fall in anti-AChR ab titer occurred during the phase of plasmapheresis, and continued immunosuppression during the followup period served chiefly to maintain her response.

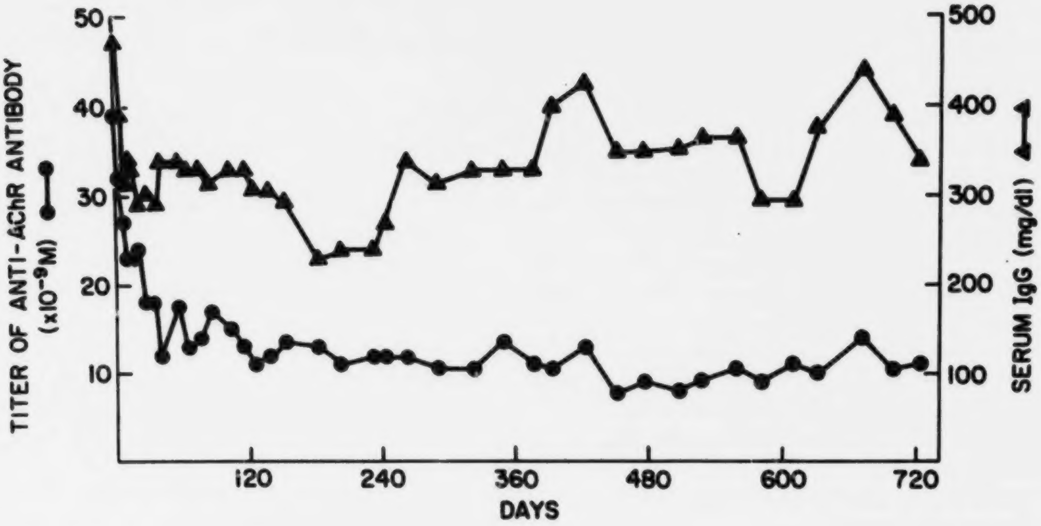
As illustrated in figure 2, three typical phenomena were found in studying anti-AChR ab titers in those patients achieving stable clinical improvement after plasmapheresis and immunosuppressive drug therapy: A) There was a rapid initial fall in anti-AChR ab titer with each plasmapheresis until a plateau was reached at a considerably lower titer (approximately 30% of the original titer). Subsequent plasmaphereses produced little overall reduction in titer, i.e., reaccumulation (resynthesis minus catabolism) of anti-AChR ab equaled the rate at which it was being removed by plasmapheresis. B) There was a disassociation between the greatly lowered anti-AChR ab titers and the relatively less depressed total IgG levels. That effect was probably produced at least in part by the replacement of normal immunoglobulin during the phase of plasmapheresis by means of gamma globulin injections. C) After cessation of the series of plasmaphereses, there was little if any increase in anti-AChR ab titers, although the total IgG levels returned to the pretreatment values (6). The ultimate selective depression of anti-AChR ab titers compared to the total IgG ab population indicates that the clones of lymphoid cells responsible for the production of the autoantibody may have been selectively depleted by the action of immunosuppressive drugs during the phase of plasmapheresis (3). Following plasmapheresis, the autoreactive lymphoid clones may have been held in check by the continued action of the immunosuppressive drugs, or by partially reestablished suppressor cell control (12). In no case has anti-AChR ab been completely eliminated from the patient's circulation.

The group of seven MG patients who suffered partial relapse when plasmapheresis was terminated received an average of 13 plasmaphereses, 2 more than those attaining stable improvement (table 4). It is instructive to note the relative inadequacy of the immunosuppressive drug therapy in all seven of them. Two received azathioprine only, and one received predominantly azathioprine alone because prednisone had to be withdrawn after a short course. One received cyclophosphamide alone during plasmapheresis, and that drug had to be withdrawn immediately afterward because of leukopenia. One was stable during followup until prednisone had to be tapered, one received only a low dose of prednisone (20 to 30 mg every other day), and one had azathioprine withdrawn when a staphylococcal arthritis developed several weeks after the conclusion of plasmapheresis. The patient who completely relapsed also was intolerant of corticosteroids and received either azathioprine or cyclophosphamide alone during her plasmapheresis. The results underscore the difficulty in adequately treating moderate to severe MG with plasmapheresis unless a full complement of immunosuppressive drugs can also be employed. Once control of the myasthenic disease process has been achieved by the combined action of plasmapheresis and immunosuppressive drugs, it must be maintained by the continuous administration of immunosuppressive medication during the followup period. To reduce the incidence of side effects, immunosuppressive drugs should eventually be tapered to the minimum dose adequate to control the patient's disease. Tapering should be undertaken only in patients who have been stable for several months after their series of plasmaphereses, and it should proceed very slowly under careful monitoring of each patient's clinical status and anti-AChR ab titer.

Five patients showed recurrence of a monotonous constellation of weakness when plasmapheresis was stopped, although they had achieved substantial improvement in their MG symptoms. They would reach a zenith of improvement during the first week following a treatment, then have a phase of stability lasting about 3 to 10 days, and then begin to experience gradually increasing weakness. Their improvement has been "maintained" with repeated plasmaphereses carried out at 10- to 30-day intervals. Four of these patients have received a mean of 48 treatments (table 4) carried out over 24 months; the fifth relapsed completely when maintenance plasmaphereses were interrupted after 28 treatments because of the development of allergy to allogenic gamma globulin, and immunosuppression was withdrawn due to side effects. In those individuals, the severity of the underlying disease process did not allow adequate containment of anti-AChR ab synthesis by the immunosuppressive drugs after the initial series of plasmaphereses. A net reaccumulation of circulating anti-AChR ab eventually rose above the level required for the symptomatic recurrence of MG. The patient's improvement has been maintained by periodically reducing the anti-AChR ab titer with plasmapheresis, so that it did not rise above a mean value of 28 percent of the original titer. After the initial series of plasmaphereses, the anti-AChR ab titer as measured just before each plasmapheresis showed no further net decline, that is, anti-AChR ab synthesis was equal to catabolism plus the amount of anti-AChR ab removed by the maintenance plasmaphereses.

Figure 3 illustrates these points in a 29-year-old woman with a 2-1/2 year history of severe MG despite thymectomy (thymic hyperplasia) within months of onset and treatment with high doses of prednisone (100 mg on alternate days). Azathioprine 3.0 mg/kg was initiated, and she received 40 plasmaphereses over 2 years; after 4 months (16 treatments) the anti-AChR ab titer had fallen to 29 percent of the original titer, and there was marked clinical improvement. Her arm-extended time increased from 0 to 2.5 minutes, her vital capacity increased from 1.9 to 4.1 liters, her pyridostigmine intake decreased from 1,620 to 240 mg daily, and her EMG decremental response decreased from 70 to 18 percent. However, 24 additional plasmaphereses carried out over the next 20 months did not produce additional clinical improvement, and her anti-AChR ab titer remained at 29 percent of the original titer despite continuation of the same immunosuppressive drug therapy.

Figure 3.--Serial determination of titer of antibody to acetylcholine receptor (anti-AChR) and serum IgG in a myasthenia gravis patient. The antibody (closed circles) and serum IgG (closed triangles) were measured at the beginning of each plasmapheresis.



In two of the five patients the status quo was interrupted and a further net reduction in titer and clinical improvement was produced by substituting cyclophosphamide 2.5 mg/kg BW for azathioprine (6). None of those patients has yet been able to stop maintenance plasmapheresis altogether; it should be possible once adequate immunosuppression is achieved.

The patient who showed no response to plasmapheresis was a wheelchair-bound 35-year-old woman whose persisting disability seemed to have an emotional basis--much pseudoweakness without true weakness was demonstrable on muscle testing, and she responded positively to placebo injection of "anticholinesterase medication." Her thymectomy 18 years previously, 1 year after disease onset, had probably produced a remission of her MG, as evidenced by a very low level of anti-AChR ab (3.81 x 10^{-9} M/L) and lack of a decremental EMG response.

From our experience with 32 moderately to severely weak MG patients treated with plasmapheresis, we conclude that plasmapheresis combined with immunosuppressive drug therapy is a powerful therapeutic modality, producing sustained clinical improvement in most MG patients. The magnitude and duration of the response to plasmapheresis probably depend upon three factors: the intensity of the underlying disease process as reflected in the speed of replacement of anti-AChR ab removed by plasmapheresis; the use of immunosuppressive drugs during plasmapheresis to bring the propensity to synthesize anti-AChR ab under control; and the use of an extended series of plasmaphereses, adjusted to the clinical response of each individual patient, so that the immunosuppressive drugs have adequate time to exert their action.

IDIOPATHIC INFLAMMATORY MYOPATHY

The idiopathic inflammatory myopathies are a heterogeneous group of acquired diseases that are thought to have an autoimmune basis because of the frequent presence of inflammation without evidence of infection. Three major types have been distinguished (13): polymyositis, a primary myopathy affecting mostly adults; dermatomyositis, a primary myopathy together with an erythematous skin rash occurring frequently in children as well as in adults, where it is sometimes associated with carcinoma; and polymyositis (and dermatomyositis) occurring in association with other diseases of presumed autoimmune pathogenesis, chiefly scleroderma (14), rheumatoid arthritis (15), systemic lupus erythematosus (16), and mixed connective tissue disease (17,18).

The main pathological characteristics of all three types of IM are degeneration, regeneration, and phagocytosis of individual muscle fibers, perivascular and interstitial inflammatory infiltration (19-21), and perifascicular atrophy (22). Other than an increased incidence of vasculitis in associated polymyositis (23), there appears to be little pathologically to distinguish between the three types of IM except factors related to severity, chronicity, and disuse atrophy (24). Most cases of childhood dermatomyositis are unique in that the basic lesion in muscle is an angiopathy with multiple thrombus formation and infarction of muscle (25-28). However, less pronounced but somewhat similar ultrastructural lesions of capillaries have been described in polymyositis (29-31).

At present, a cell-mediated autoimmune reaction is widely held to be responsible for the production of IM. That belief is based on the experimental production of polymyositis in animals by immunizing with skeletal muscle in Freund's adjuvant and the finding of cytotoxic reactivity by lymphocytes from IM patients against cultures of skeletal muscle cells (32, 33). However, there are three recent reports (6,34,35) of four cases of IM refractory to immunosuppressive drug therapy which improved with plasmapheresis, suggesting the involvement of a circulating factor such as autoantibody or immune complexes in the production of the muscle lesions. The cases included one primary polymyositis (6), one of polymyositis in association with systemic lupus erythematosus (34), one of polymyositis in association with mixed connective tissue disease (35), and one of polymyositis in association with scleroderma (6).

A possible explanation for the efficacy of plasmapheresis in the treatment of IM is provided by the finding of intramuscular deposits of immunoglobulins and complement by means of direct immunofluorescence staining. Whitaker and Engel (36) found IgG and/or IgM in skeletal muscle blood vessel walls of 17 of 39 patients with IM. In seven patients with active dermatomyositis and immunoglobulin deposits, there were also vascular deposits of C3. Only rarely did the vessels with immunoglobulin deposits have concomitant inflammation. Vascular immunoglobulin deposition was found in 9 out of 11 cases of childhood dermatomyositis, but only in 3 out of 12 cases of adult dermatomyositis, 2 out of 7 cases of polymyositis, and in 3 out of 7 cases of polymyositis with associated collagen-vascular disease. The finding of frequent vascular deposits of immunoglobulins in childhood dermatomyositis fits well with the morphologic documentation of vascular lesions in this disorder (25-28), and strongly suggests that an autoimmune vasculopathy may be the etiologic factor of paramount importance. Heffner et al. (37) found evidence of immune complex deposition in the microvasculation of skeletal muscle in two out of three cases of dermatomyositis and in two out of three cases of IM associated with systemic lupus erythematosus, as did Ringel et al. (31) in a case of IM associated with hyperglobulinemic purpura. In addition, Behan and Behan (38) and Ringel et al. (31) have presented evidence for circulating immune complexes in IM.

Since Whitaker and Engel found vascular immunoglobulin deposits in only 31 percent of cases of adult IM, other autoimmune mechanisms in addition to vasculopathy may play a role in such patients. Von Kiraly et al. (39) found immunoglobulins bound to the sarcolemma of skeletal muscle in cases of IM. In a study of skeletal muscle biopsies from 45 patients with collagen-vascular diseases, Oxenhandler et al. (40) could demonstrate sarcolemma-basement membrane immunoglobulins in 29 cases and vessel wall deposition in 15 cases. In 30 patients with polymyositis associated with scleroderma, Klug et al.(41) found sarcolemmal deposition of immunoglobulins in 14 cases, and vascular deposition in 24 cases. Their results suggested a progression from the deposition of IgM and complement in capillary and arterial vessel walls in the earliest lesions to the deposition of vessel wall and sarcolemmal IgG in the most advanced cases. Judge et al. (42) reported florid sarcolemmal deposition of IgG without complement or inflammation in a patient with marked weakness and autoimmune glomerulonephritis. Some authorities, however, contend that sarcolemmal staining for immnoglobulins in IM is nonspecific (37).

Our series of IM patients treated with plasmapheresis has now reached 14 cases (table 5) and they have received a total of 197 plasmaphereses. With the exception of two cases of mild polymyositis occurring in association with scleroderma (cases 5 and 9), all patients had been treated with moderate- to high-dose prednisone therapy, with unsatisfactory results, prior to receiving plasmapheresis, and five cases had been treated with cytotoxic drugs as well. Prednisone, 50 mg daily, was begun in cases 5 and 9, and the preexisting level of prednisone

Table 5.--Patients receiving plasmapheresis therapy for inflammatory myopathy

Case	Age (Years)	Sex	Diagnosis	Previous Therapy*	Number of Exchanges	CPK (IU/L) Initial	CPK (IU/L) Last	Change in Strength
1	22	F	Polymyositis	pred, az, cy, mtx, 6-MP	8	390	195	improved
2	31	F	Scleroderma	pred, az	34	94	20	improved
3	33	F	Scleroderma	pred	6	39	32	no change
4	38	F	Polymyositis	pred	18	1092	14	improved
5	37	F	Scleroderma		22	466	39	improved
6	30	F	Scleroderma	pred	26	219	34	improved
7	15	F	Dermatomyositis	pred	6	155	30	(deceased)
8	30	M	Dermatomyositis	pred, az, hmp	21	1540	125	improved
9	35	F	Scleroderma		10	90	22	improved
10	56	F	Dermatomyositis	pred, mtx	12	52	24	improved
11	46	F	Polymyositis	pred, cy, mtx	11	250	62	improved
12	29	M	Dermatomyositis	pred	6	282	42	improved
13	6	F	Dermatomyositis	pred	11	19	6	improved
14	57	M	Polymyositis	pred	6	1645	957	improved

* pred = prednisone, az = azathioprine, cy = cyclophosphamide, mtx = methotrexate, 6-MP = 6-mercaptopurine, hmp = high-dose methylprednisolone.

dosage (30 to 80 mg daily) was continued in the others. Prednisone had been discontinued in cases 1 and 3 because of lack of efficacy and intolerable side effects, and it was not reinstituted. All patients received an alkylating agent during plasmapheresis therapy, cyclophosphamide, 2.5 mg/kg BW/day in cases 1 through 12 and 14, and chlorambucil, 0.1 mg/kg BW/day in case 13. In cases 2 and 8 initially an attempt was made to continue the patient's preexisting azathioprine therapy instead of administering cyclophosphamide during plasmapheresis. In both instances, cyclophosphamide was substituted for azathioprine after the first six plasmaphereses because of the lack of a satisfactory clinical response (6). In animals, cyclophosphamide has been shown to be highly effective in inhibiting the rise in antibody titer that otherwise occurs following plasmapheresis (43); it presumably exerts a strong cytotoxic action upon clones of lymphocytes when they proliferate in preparation to synthesize more antibody.

Cyclophosphamide has not generally been found to be an effective therapeutic agent when used in the treatment of steroid-refractory IM. For example, four out of four such patients reported by DeVere and Bradley (20) expired. Of six IM patients treated with cyclophosphamide by Bohan et al. (21), of whom four also received prednisone, only two showed a modest improvement. Fries et al. (44) found no improvement in four IM patients treated with cyclophosphamide by itself.

Three patients (cases 5, 6, and 9) were treated primarily for their scleroderma and had only mild weakness and fatiguability prior to plasmapheresis. In all three, the weakness and fatiguability were completely resolved, and their creatinine phosphokinase (CPK) was normalized. The two other scleroderma patients (cases 2 and 3) had severe generalized weakness, and one of them (case 3) left the study for personal reasons without an adequate trial of therapy. Case 2, reported in detail elsewhere (6), had had bulbar and respiratory failure, being respirator-dependent for 6 months, events which carry an exceedingly poor prognosis when they occur in the context of scleroderma. At present she has only mild residual weakness, her CPK is normal, and she has been back to work as a nurse for 8 months. In Rose and Walton's series (45) of 89 polymyositis patients, three out of four cases with associated scleroderma expired despite high-dose prednisone therapy, and the fourth was unimproved. Of DeVere and Bradley's eight cases of polymyositis in association with scleroderma, three died within 14 months of the onset of polymyositis (20). Two cases treated by Currie and Walton (46) did not improve with prednisone and cytotoxic drug therapy, and Winkelmann et al. (47) found the coexistence of scleroderma and IM to be associated with a high mortality.

All four of our cases of primary polymyositis (cases 1, 4, 11, and 14) have manifested gradually increasing strength and falling CPK levels. Figure 4 illustrates the rapid normalization of CPK levels with 18 plasmaphereses carried out over 106 days in case 4, a 38-year-old female who was thought to have muscular dystrophy because she developed severe weakness that was refractory to moderate-dose prednisone (40 to 60 mg/day) administered for 5 months. Treatment of case 1 had to be interrupted because of allergic reactions to gamma globulin injections (6), and cases 11 and 14 are still being actively treated. Four out of five dermatomyositis patients are stronger, with lowered CPK values, and are still having plasmaphereses. One dermatomyositis patient (case 7) suffered respiratory failure and aspiration pneumonia before her rapidly progressing disease was controlled (as manifested by partial healing of her extensively ulcerated skin and normalization of serum muscle enzymes). She expired of sepsis just 7 weeks after admission to the hospital. Direct immunofluorescence staining of muscle biopsies done in four of the five dermatomyositis patients showed sarcolemmal deposition of IgG in cases 7, 8, and 12 (figure 5) and blood vessel wall deposition of IgM and IgG in case 13 (childhood dermatomyositis, figure 6). In addition, the only one (case 14) of the four polymyositis patients biopsied was weakly positive for sarcolemmal IgG.

Figure 4.--Serial determination of serum creatinine phosphokinase activity in idiopathic inflammatory myopathy, case 4. CPK was measured at the beginning of each plasmapheresis (closed circles) and during the followup period without plasmapheresis (open circles).

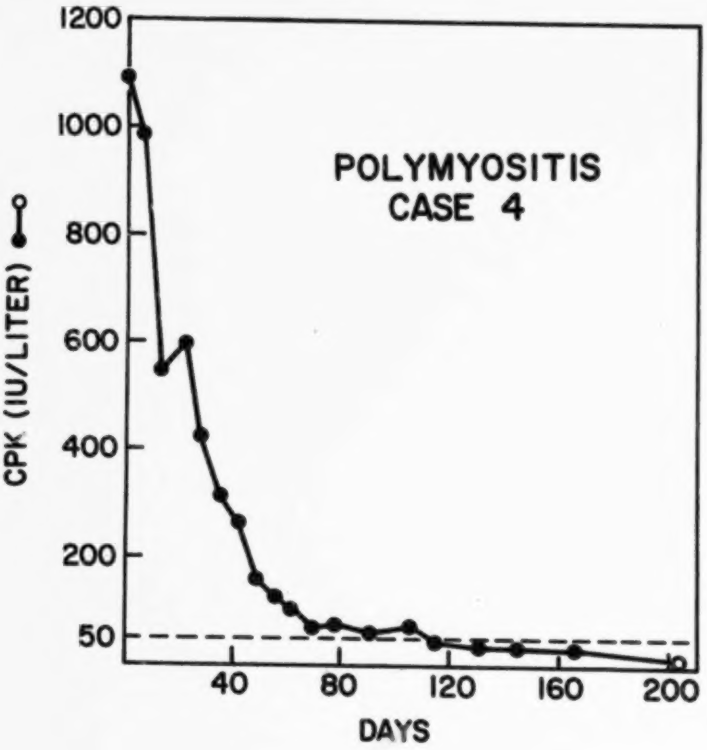


Figure 5.--Direct immunofluorescence staining of muscle biopsy from idiopathic inflammatory myopathy, case 12: (a) stain with fluorescein conjugated goat antihuman IgG, (b) stain with fluorescein conjugated goat antihuman albumin.

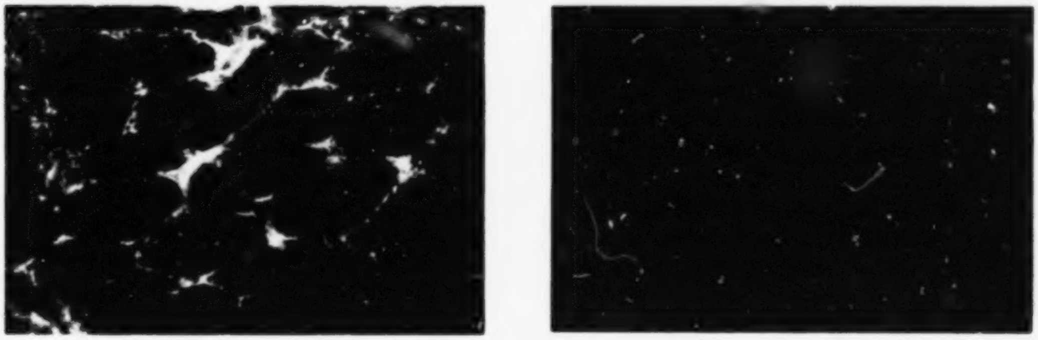


Figure 6.--Direct immunofluorescence staining of muscle biopsy from idiopathic inflammatory myopathy, case 13: (a) stain with fluorescein conjugated goat antihuman IgG, (b) stain with fluorescein conjugated goat antihuman albumin.

In conclusion, plasmapheresis combined with cyclophosphamide therapy appears to be a powerful new treatment in the management of corticosteroid-refractory IM, and our results suggest that circulating autoantibodies or immune complexes may play an important role in the pathogenesis of IM.

MULTIPLE SCLEROSIS

Multiple sclerosis is a demyelinating disease of undetermined etiology affecting the central nervous system. In 50 percent of cases it leads to major disability within a few years of onset (48). There is no generally effective therapy, although some recent studies suggest that the disease process can be slowed by intensive immunosuppression (49). A considerable body of indirect evidence implicates immunological processes in the pathogenesis of MS. To begin with, the structure of the MS lesion (plaque) suggests a hematogenous origin. Small plaques are clearly centered about central veins; larger plaques arise by extension and confluence of smaller plaques. The histologically least active, and therefore oldest, area of plaque is closest to the central vein; conversely, the outermost edge is the most active zone of myelinolysis. Demyelination in active plaques is complete, or most complete, centrally, and least complete peripherally (50).

MS brain tissue contains increased amounts of IgG, but normal amounts of albumin (51). Since IgG is a much larger molecule than albumin, the accumulation of immunoglobulin in MS brains cannot be on the basis of simple diffusion through a leaky blood-brain barrier. The IgG, as well as complement, is preferentially localized to the peripheral zone of active demyelination in active MS plaques (52-54), and its concentration correlates positively with the amount of histologically defined inflammation within the plaque (55). IgG at the plaque margin appears to be bound to myelin (53) and can only be washed out at pH 2, a condition under which immune complexes dissociate (54). By contrast, IgG located centrally within plaques, where the putative myelin and oligodendroglial antigens are greatly reduced to absent, is easily removed by washing with saline solution (55,56). Plasma cells frequently occur in active plaques, suggesting the local production of antibody (57). The concentration of IgG relative to albumin is increased in the cerebrospinal fluid (CSF) from all cases of active MS (58), more so in prolonged, severe cases (59). Although elevated antibody titers toward a number of common human pathogenic viruses are found with increased frequency in MS CSF (60,61), the bulk of the IgG in MS CSF is not absorbable with antigen from those viruses (61). Antibody toward myelin basic protein, the eliciting antigen in the production of experimental allergic encephalomyelitis in animals, has recently been found in MS CSF (62). However, the antigenic specificities of the increased MS CSF immunoglobulins are, for the most part, yet to be determined.

Antibodies toward myelin basic protein have not been detectable by radioimmunoassay in MS sera (62-65), nor have B-lymphocytes with specificity toward myelin basic protein been detectable in the circulation of MS patients (66). If demyelination in MS is produced by an autoantibody directed against myelin or oligodendroglia, it is therefore likely that its specificity is for an antigen other than myelin basic protein. On the other hand, antimyelin antibodies are found in MS sera by immunofluorescence techniques (67), and complement-fixing antibodies with specificity toward a water-soluble component of MS brain have been reported to be present in MS sera (68). More recently, antibodies with specificity toward isolated oligodendroglia were found in the sera of 19 of 21 MS patients (69). The majority of sera from patients with active MS demonstrate demyelinating activity when applied to myelinated central nervous system tissue cultures together with complement (70,71). The demyelinating factor is a thermolabile immunoglobulin and does not affect cultures containing peripheral myelin (72). This *in vitro* demyelinating activity is also found in concentrated MS CSF and saline eluates made from plaques, but not from normal-appearing MS white matter (73). Unconcentrated CSF from MS patients in acute relapse can produce demyelination *in vivo* when injected adjacent to tadpole optic nerves (74), and MS IgG produces complement-dependent blocking of neuroelectric activity in the frog spinal cord, especially during acute exacerbation (75).

In the majority of MS patients, the first phase of the disease is characterized by fluctuations in clinical symptomatology which makes evaluation of therapy difficult. Most patients eventually enter a progressive phase in which the discreteness of individual attacks becomes blurred, there is insidious worsening, and "long-term" symptoms predominate (76). In order to allow a meaningful assessment of the therapeutic response to plasmapheresis and immunosuppressive therapy in a small group of MS patients, eight individuals in the progressive phase of the illness were selected for study (table 6). Up to 20 plasmaphereses were carried out at 1- to 2-week intervals. Immunosuppressive medication consisted of azathioprine, 2.5 to 3.0 mg/kg BW daily, and prednisone, 100 mg daily for 4 days following each plasmapheresis. No patient was receiving immunosuppressive medication at the time of entry into the study.

Seven of the eight patients (all but case 6) felt improved very early, after their first to third plasmapheresis. The subjective improvement was accompanied by probable neurologic signs of objective improvement, and such signs were definitely present by the 6th to 10th plasmapheresis (table 7). The bulk of the neurologic disability in those chronic patients, however, was not influenced by plasmapheresis. There were three discrete episodes of clinical relapse during the serial plasmapheresis. In case 3, it occurred in association with lumbar strain, and in cases 4 and 5, during physically stressful vacations. In cases 3 and 5 there was also a prolongation of the treatment interval (to 20 and 18 days, respectively) and the inadvertent omission

Table 6.--Patients receiving plasmapheresis therapy for multiple sclerosis

Case	Age	Sex	Duration (Years)	Major Involvement	Number of Exchanges	Interval (Weeks)	Response	Condition During Followup	Interval (Weeks)
1	33	F	12	Paraparesis, sensory, ocular	20	36	Improved	Unchanged	42
2	33	F	4	Paraparesis, tremor, sensory, ocular	20	35	Improved	Unchanged	42
3	46	M	6	Paraparesis, sensory, ocular	20	28	Worse	Unchanged	26
4	43	F	9	Paraparesis	20	29	Improved	Unchanged	25
5	39	F	10	Paraparesis, sensory	20	26	Improved	Relapsed	27
6	51	F	6	Tremor, dementia	10	12	Improved	Unchanged	25
7	40	F	3.5	Sensory	14	15	Improved	Relapsed	23
8	42	F	0.6	Tremor, sensory, monoparesis	20	20	Improved	Improved	2

Table 7.--Clinical response to plasmapheresis by multiple sclerosis patients

Case	Subjective	Objective
1	↓urinary frequency and urgency Improved gait, more endurance ↓numbness of hands Disappearance of band on chest	↑position sense hands and feet Gait narrower based and steadier ↑strength of legs
2	↓numbness Improved speech and emotional control ↓urgency and frequency of bladder and bowel ↑mobility ↓shaking of body	↑position, vibratory, pain, and light touch sensation ↑strength ↑balance, ↓ataxia
3	↑ weakness of legs	↑weakness of legs
4	Improved gait and vitality Better balance	↑strength of left arm and leg ↑position sense in legs
5	↑ mobility and standing	↑strength of legs ↑position sense in legs
6	No change	↑strength in legs
7	↓ numbness and tingling ↑ balance	↑position, vibratory, pain, and light touch sensation Improved gait
8	↓numbness Disappearance of tight band about legs and chest Improved gait and vitality	↑position, vibratory, pain, and light touch sensation ↓ataxia ↑strength

of azathioprine medication. The increased disability was eventually reversed with subsequent plasmaphereses in cases 4 and 5, but not in case 3. Six of the eight patients were stable during followup after termination of their series of plasmaphereses (table 6). Case 5 had a severe exacerbation of weakness occurring about 3 weeks after the end of her series of plasmaphereses; it was only partly reversible with high-dose prednisone therapy (200 mg daily). Case 7 relapsed gradually about 4 months after her plasmaphereses.

H. S. Panitch and K. P. Johnson studied the cerebrospinal fluid of seven of the eight patients before and after the entire series of plasmaphereses. In no instance were increased numbers of white blood cells present. In six out of seven cases the total IgG concentration decreased, and in one case the number of oligoclonal bands decreased and the test (62) for antimyelin basic protein antibodies changed from positive to negative. Auditory and visual evoked potentials were also measured before and after the entire series of plasmaphereses in seven of the eight patients. There were three instances of improvement and three of worsening.

Sera taken before the first and tenth plasmaphereses, and several weeks after the last plasmapheresis, from the eight MS patients and from eight other patients with autoimmune disease also receiving plasmapheresis were assayed for demyelinating activity by M. B. Bornstein. Neither group showed significant demyelinating activity.

Plasmapheresis combined with immunosuppressive drug therapy is effective in lowering autoantibody titers in diseases of known autoimmune pathogenesis, such as myasthenia gravis. Although only modest clinical improvement was produced by applying the treatment to MS patients, it must be considered that in chronic MS a point must come when extensive myelin loss, axonal loss, and the buildup of gliosis within plaques produces an irreversible lesion. Our results suggest that blood-borne factors, possibly autoantibodies, may play a role in the pathogenesis of the MS plaque. Plasmapheresis is not effective in reversing the signs of advanced, longstanding MS. Combined with appropriate immunosuppressive therapy, it might have a place in the treatment of exacerbations early in the course of the disease. A randomized, prospective study of this issue seems warranted.

MYASTHENIC SYNDROME

The myasthenic syndrome was first described by Eaton and Lambert in a patient with bronchogenic carcinoma (77), but it also can occur in patients without demonstrable cancer. The characteristic electromyographic features of the disease are a low amplitude of the muscle potential evokable by supramaximal stimulation of the motor nerve, a decrement in the evoked potentials at slow rates of stimulation, and an increment in the evoked potentials at tetanic rates. Electrophysiological studies have shown a decrease in the release of acetylcholine quanta from the nerve terminal to account for the faulty neuromuscular transmission in the myasthenic syndrome (78). A similar defect has been produced in the frog nerve-muscle preparation by application of an extract from a bronchogenic carcinoma removed from a patient with myasthenic syndrome (79). Thus, a circulating factor released by the neoplasm may produce the myasthenic syndrome in some patients with cancer. In others, it is conceivable that antibodies directed against tumor-associated antigens could produce the myasthenic syndrome by crossreacting with antigens represented on cholinergic presynaptic membranes. The finding of a beneficial clinical response to corticosteroid therapy in some noncancerous cases of the myasthenic syndrome (80,81) suggests that pathogenic immune mechanisms might be operative.

Our patient, a 43-year-old male with a 2.5-year history of noncancerous myasthenic syndrome, was treated with nine plasmaphereses without immunosuppressives (for clinical details, see reference number 82). A substantial clinical improvement could be documented by serial electromyographic studies before, during, and after his series of plasmaphereses (table 3). Plasmapheresis resulted in an almost threefold increase in the maximum amplitude of the evoked muscle potential recorded from the abductor pollicis brevis upon supramaximal stimulation of the motor nerve, and a corresponding reduction of the facilitation recordable with tetanic stimulation (50 Hz) of from 28- to 45-fold to 14-fold. At followup 1 year later there was modest clinical regression, the maximum amplitude of the evoked muscle potential had diminished to less than the preplasmapheresis value, and the amount of facilitation had increased to 41-fold (table 8).

Table 8.--M-wave in the myasthenic syndrome during a series of plasmaphereses

Date	Number of Plasmaphereses	Initial Amplitude	Maximum Amplitude (50/second)	Facilitation
Day 1	0	0.26 mV	7.2 mV	28 times
Week 2	3	0.28 mV	12.5 mV	45 times
Week 6	7	0.52 mV	10.5 mV	20 times
Week 7-/2	9	0.72 mV	10.0 mV	14 times
Month 13	9	0.22 mV	9.0 mV	41 times

This case study provides evidence for the possible role of a circulating factor in the pathogenesis of the myasthenic syndrome. Studies in two other myasthenic syndrome patients, as well as animal transfer studies using IgG purified from the patients' plasma, are in progress.

ADDENDUM

Through April of 1980 we have treated 50 patients with myasthenia gravis, 33 patients with idiopathic inflammatory myopathy, and 3 patients with the Eaton-Lambert syndrome. Our experience with this larger patient material conforms to the results previously reported. In multiple sclerosis, one additional patient with subacute disease showed pronounced improvement with a different treatment schedule consisting of twice-weekly plasmapheresis for 4 weeks, together with cyclophosphamide, 3 mg/kg body weight, and prednisone, 1 mg/kg body weight daily.

We also now have experience treating five patients with the acute Guillain-Barre syndrome. Each patient received twice-weekly plasmapheresis for 2 to 3 weeks, without any prednisone or other immunosuppressive drugs. Three out of four severely paralyzed patients with tetraparesis and bulbar involvement made a spectacular recovery within weeks, commencing with the first plasmapheresis. The fourth patient showed only modest upper extremity and bulbar improvement, and remained paraplegic. A fifth patient, with chiefly facial diplegia showing complete electromyographic denervation, did not show improvement of his facial muscles but did respond in other, less involved areas.

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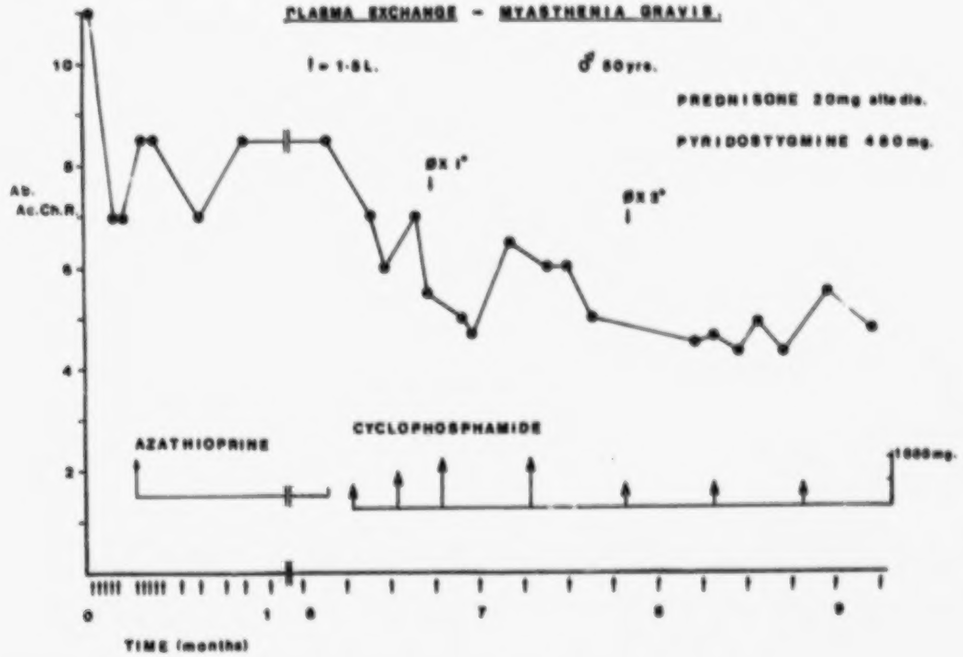
DISCUSSION

Dr. Branda: Before opening general discussion of Dr. Dau's paper, there was a gentleman who wanted to show a couple of slides about plasmapheresis in myasthenia.

Dr. Roger Bucknall: I would like to comment on this question of immunosuppression. I have recently had the opportunity, while working in Bristol, England, to study the effects of plasmapheresis and cytotoxic therapy on a patient with myasthenia gravis. We were able to measure the acetylcholine receptor protein antibody, but we also assessed the patient's response to a primary immunogen. We gave primary and secondary immunizations with the bacteriophage ø x 174; and if I could just show these slides quickly--



This is a 50-year-old man [who] had had myasthenia for several years, and in spite of being on steroids and large doses of anticholinesterase therapy, his disease was progressing. He had also had a thymectomy. He had generalized muscle weakness, including weakness of the respiratory muscles. It was on this basis that we decided to plasma-exchange him, initially on a daily basis for 2 weeks, then fortnightly for a period of 6 months, and thereafter on a weekly basis.

The antibody to acetylcholine receptor protein was measured by the I-125 bungarotoxin assay, and this was performed by Dr. Roger Dawkins in Perth, Australia.

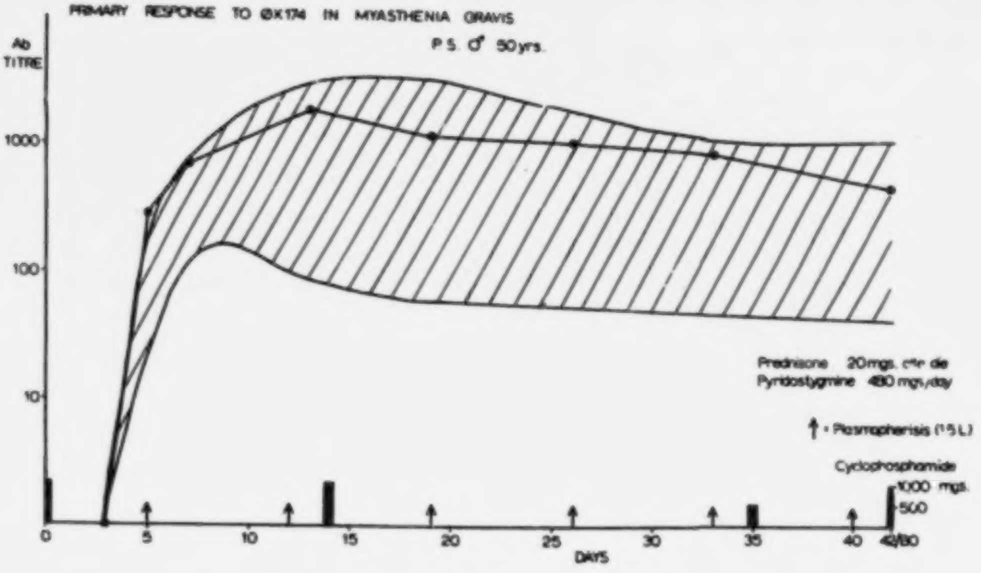
And you can see that following the first few plasmaphereses, there was a very abrupt reduction in the antibody titer, and this coincided with a marked improvement in his clinical condition.

However, he subsequently relapsed somewhat. We introduced azathioprine, which he remained on for about 6 months. During this time he was reasonably well controlled, but then subsequently his disease became clinically worse.

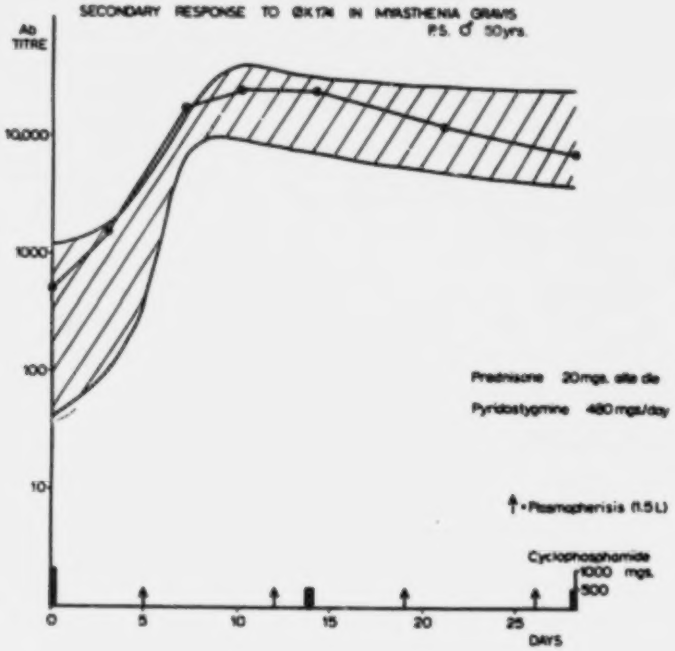
At this time, we decided to institute cyclophosphamide. This was given in intravenous boluses initially, at weekly intervals, starting with 500, going up to 1,000 mg. At this time, the intravenous injections were given the day after plasmapheresis to try to catch the rebound effect, which we had been hearing about. You can see that at this time, there was quite a marked reduction in the titers of the autoantibody; and this again corresponded with a marked improvement in his clinical condition.

Now, over the next 2 months, we performed two immunization procedures, giving intravenous bacteriophage Ø x 174, this test having been developed in Bristol by Dr. Verrier Jones before he came to Chicago.

The next two slides show the response to immunization with this antigen. Would you put the next slide on, please?



This is the response to primary immunization. The hatched area corresponds to normal controls. This is the mean, plus-or-minus one standard deviation. You can see there is quite a wide scatter but that the patient's response fell well within that normal range. This was in spite of having weekly cyclophosphamide and plasmapheresis. The next slide shows the response to secondary immunization.



In our experience it is a much better way of assessing the immune response. You can see that the controls fall into a more neatly packed group. But again, we are able to show that his response to immunization was entirely normal.

So what we have shown is that although we have got a reduction in the autoantibody titer, there is no overall suppression of the immune response.

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PLASMA EXCHANGE FOR SEVERE RHESUS (Rh) DISEASE

John Graham-Pole, M.D., M.R.C.P.
University Hospitals of Cleveland
Cleveland, Ohio

Michael Willoughby, M.D., M.C. PATH.
Queen Mother's Hospital
Glasgow, United Kingdom

INTRODUCTION

The use of a cell separator allows rapid and frequent exchange of large plasma volumes without great discomfort to the patient and without producing deficiency of circulating albumin or immunoglobulins. It is ideally suited to treating immunoproliferative disorders of limited duration, whose pathogenesis is directly related to the production of abnormal antibodies.

The amount of circulating maternal anti-D antibody relates directly to the severity of fetal hemolysis (9),and attempts have been made for more than a decade to lessen severity of erythroblastosis by manual plasmapheresis of small volumes of maternal plasma (4,11). Those attempts have had limited success, but a recent series using manual exchange of 1 to 4 liters per week achieved a 61 percent fetal survival (6). However, there were only 3 survivors of 12 who required therapy before 28 weeks of gestation.

We have used intensive plasma exchange with an Aminco cell separator for 12 women with exceptionally severe Rh isoimmunization developing early in pregnancy and have tried to answer the following questions:

1. Can circulating maternal anti-D be removed faster than it reaccumulates?
2. Does such removal result in decreased fetal hemolysis, as measured by amniotic fluid spectrophotometry?
3. Can intensive plasma exchange without intrauterine transfusion (IUT) save affected infants when the mother has had previous Rh stillbirths?

Details of some of these studies have been previously published (8).

PATIENTS AND METHODS

Obstetric and serological details on nine subjects are shown in table 1. Data on three subjects are excluded as they had three or fewer exchanges before the procedure was abandoned.

Table 1.--Plasma exchange for Rh diseases: Obstetric and serological data on nine treated patients

Patient	Age	Obstetric History			Serology		Antibody Present
		Rh Unaffected Survivors	Rh Affected Survivors	Rh Deaths	Blood Group	Husband's Genotype	
C.G.	31	1	1	3	O-	O+cDE/cde HE	D
R.M.	30	1	1	3	AB-	O+CDe/cDE HO	CD
R.L.	31	0	1	0	O-	O+CDe/cde HE	D
L.S.	30	1	0	2	A-	O+CDe/CDe HO	D
J.T.	32	1	2	1	O-	O+CDe/cDE HO	D
S.W.	27	1	0	2	O-	O+CDe/cde HE	D
A.B.	29	1	1	1	O-	O+CDe/CDE HO	D
E.P.	33	1	1	2	B-	O+CDe/cDE HO	D
M.G.	38	1	1	4	A-	A+CDe/CDe HO	CD
		7	8	16			

Rh = Rhesus isoimmunization

HE = Heterozygote for 'D'

HO = Homozygote for 'D'

Criteria for Selection

The decision to start plasma exchange was made when a combination of obstetric history, Coombs' titer, anti-D IgG level, and amniotic fluid spectrophotometry suggested that the fetus was unlikely to survive with conventional management.

Obstetric History

Of a total of 31 pregnancies lasting at least 28 weeks, 24 (77%) were complicated by Rh isoimmunization, and 16 (66%) of those ended in fetal loss. Only one mother had not lost an Rh baby, and she had been immunized as a child with massive blood transfusions. Six fathers were homozygous for the Rh (D) antigen.

Maternal Antibody Level

Rh antibodies were measured by indirect Coombs' test and anti-D IgG concentration. The latter generally correlates well with the severity of hemolysis (9). All but one subject had early pregnancy levels above 1.5 mcg/ml IgG, mostly much higher, suggesting severe Rh disease independent of the obstetric history (7).

Amniocentesis Findings

Eight of the nine had amniotic fluid spectrophotometry performed by Liley's method (10) before starting plasma exchange. Though definite predictions cannot be made before 25 weeks, amniocentesis probably provides the best index of severity and is essential for monitoring disease progress. All eight had levels above 0.1 optical density (OD) units; five had levels greater than 0.2 units, which is equivalent to the IUT zone at 34 weeks.

Plasma Exchange Technique

Volumes ranging from 2.5 to 7.0 liters of plasma were exchanged for reconstituted freeze-dried plasma or purified protein fraction, according to availability. When the latter was used, 500 ml of fresh-frozen plasma were given at the end to restore clotting factors. Heparin was given to a total dose of 10,000 units as anticoagulant, and 1 g calcium gluconate was given intravenously at the start and at the end of the procedure when freeze-dried plasma was used. The volume exchanged increased steadily through the study period. The first three subjects were treated less intensively because our initial aim was to control fetal hemolysis until IUT was technically feasible. Because plasma exchange was well tolerated and was unsuccessful when followed by IUT, more intensive exchange (10-20 l/wk) was subsequently tested alone.

RESULTS

Outcome of Pregnancy

Table 2 shows details of exchanges and IUT's in nine pregnancies and their eventual outcomes. A mean of 26 plasma exchanges were performed removing 106 liters over 12 weeks. In spite of this intensive program only three infants survived (33%), though a fetal salvage rate of zero was predicted without this early intervention. There were three other live births at 29, 33, and 37 weeks, two died of prematurity and Rh disease and the other of respiratory distress syndrome in spite of good control of her Rh problem. Four babies had IUT's, and all died as a direct result (three traumatic, one premature labor). Three mothers delivered live Rh-positive infants at 37, 36, and 34 weeks, having been given plasma exchanges from 20, 20, and 22 weeks, respectively. Their infants required 2, 1, and 0 exchange transfusions, and they remain healthy.

Table 2.--Plasma exchange and intrauterine transfusions for Rh disease

Patient	Plasma Exchanges		Gestation Period	I.U.T.	Outcome	Gestation
	Number	Total Volume (litres)	(weeks)	(Gestation Time)		(weeks)
1. C.G.	14	26.7	20 - 27 (8)	27, 29, 31	IUD	31
2. R.M.	13	24.5	19 - 27 (8)	27	NND	28
3. R.L.	28	73.0	20 - 28 (9)	28	IUD	28
4. L.S.	35	164.0	20 - 36 (17)	nil	live	36
5. J.T.	19	80.0	27 - 37 (10)	nil	NND	37
6. S.W.	42	237.0	20 - 35 (16)	nil	live	36
7. A.B.	13	62.0	22 - 28 (7)	nil	live	34
8. E.P.	30	152.0	21 - 28 (8)	27, 29	IUD	29
9. M.G.	40	230.0	16 - 33 (13)	nil	NND	33
Means	26	105.9	(12)			32

IUT = Intrauterine Transfusion NND = Neonatal Death

IUD = Intrauterine Death

Effects on Mothers

Three women withdrew from the study shortly after the start, being unable to withstand the stress of the prolonged therapeutic program. The remainder tolerated the procedure well though one required an arteriovenous shunt.

Effects on Rh Antibody and Amniocentesis Findings

Table 3 shows the changes in maternal antibody and amniotic fluid optical density. There is a wide scatter in pretreatment maternal anti-D level (0.6-55.7 μg/ml), and the initial level was not helpful in predicting the success of plasma exchange. Though the mean value fell significantly over the treatment period, the difference in the falls in anti-D between the three successful and the six unsuccessful pregnancies was not significant (53% and 50%, respectively). Amniotic fluid OD values correlated poorly with maternal antibody, the three highest initial OD values being in mothers with the lowest circulating anti-D levels. The peak amniotic fluid OD level reached in the current pregnancy was, however, significantly lower than the peak in the previous (no plasma exchange) pregnancy. Mean OD changes in the three successful and six unsuccessful pregnancies were -32 percent and +74 percent, respectively, showing the value of regular amniocentesis in monitoring response and the evidence of continuing target organ disease in spite of long-term control of antibody levels.

Table 3.--Plasma exchange for Rh disease: Effect on maternal antibody and amniotic fluid bilirubin

Subject	Coombs' Titer		Anti D IgG (μg/ml)		Maximal Optical Density (A450nm)	
	Pre	Post	Pre	Post	Current Pregnancy	Previous Pregnancy
R.L.	512	16	55.7	4.2	.330	.367
R.M.	512	512	28.3	13.5	.384	.285
E.P.	256	16	15.5	3.8	.547	Unknown
S.W.	64	16	12.2	1.5	.254	.660
L.S.	64	64	10.0	7.0	.169	.380
C.G.	512	64	5.5	1.5	.344	.735
M.G.	64	128	4.8	2.5	.270	.510
J.T.	128	32	1.9	2.5	.221	.294
A.B.	64	16	0.6	0.36	.280	Unknown
Means			14.9	4.1	.282 (n=7)	.462 (n=7)
			$t = 2.16$		$t = 2.54$	
			$p < 0.05$		$p < 0.05$	

Figures 1 and 2 plot graphically the changes in Rh antibody (eight subjects) and OD values (nine subjects) during the period of plasma exchange. A semilog scale is used with Liley's OD zone, as shown in figure 2. The discontinuous lines represent subjects treated less intensively early in the study.

Efficacy of Plasma Exchange

Though very large amounts of antibody were removed from the mothers, the overall fetal salvage is disappointing. We have tried to measure the efficacy of plasma exchange both in depleting antibody and in arresting the disease.

Figure 3 combines results from three mothers and shows a highly significant correlation between the volume of plasma exchanged and the fall in antibody, a 70 percent fall resulting from a 6-liter exchange. Figure 4 shows similar data for all nine subjects (203 values), according to whether less than 3, 4 to 5, or more than 5 liters were exchanged. The same pattern is seen, the mean percentage fall in antibody being significantly greater in group 2 than in group 1 and in group 3 than in group 2.

The optimal amount of plasma that should be exchanged per run is unclear. Figure 5 shows antibody concentrations in successive collection bags from two subjects who had mean exchanges of 5.0 and 5.8 liters. The concentration fell significantly from bag 1 to bag 3, though the third bag still contained useful amounts. Continuing beyond 6 liters at a single procedure is clearly of limited value.

Figure 1.--Changes in rhesus antibody level during plasmapheresis (eight patients)

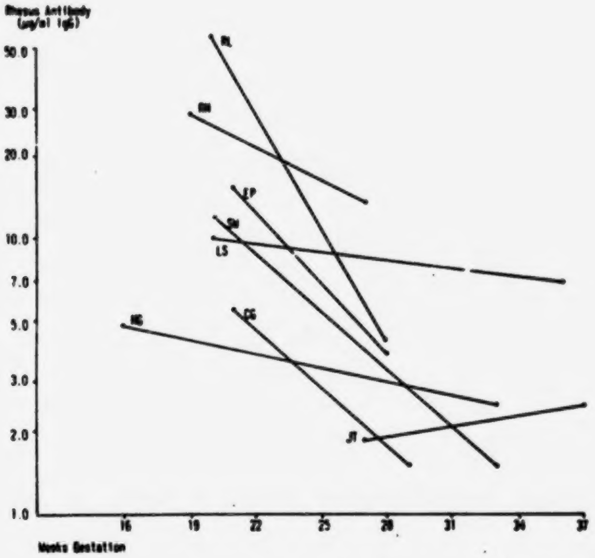


Figure 2.--Changes in amniotic fluid optical density during plasmapheresis (Liley's zones are shown: The first three less intensively treated patients are shown by discontinuous lines.)

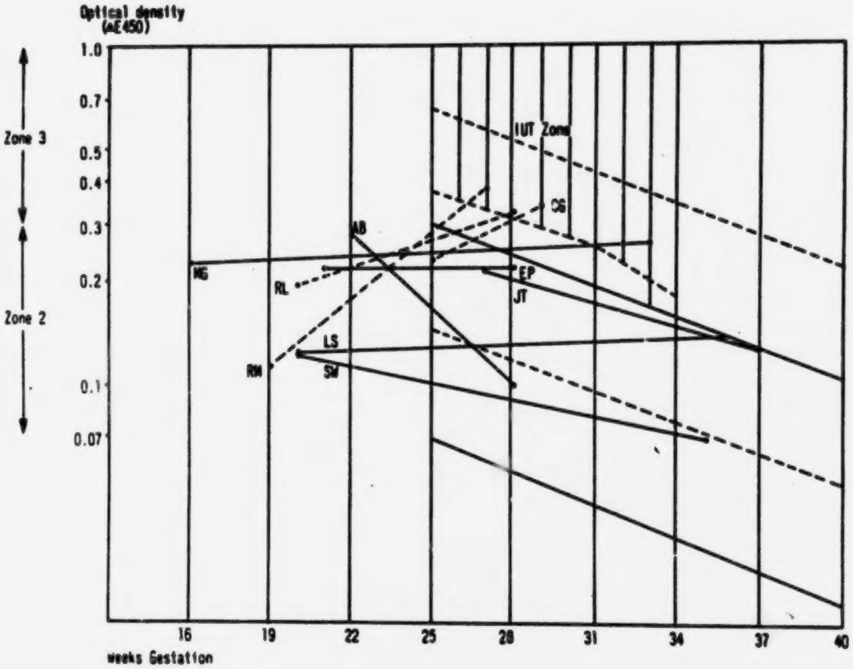


Figure 3.--Fall in antibody level related to plasma volume exchanged (patients CG, EP and MG) (regression equation y = 7.84x + 20.13)

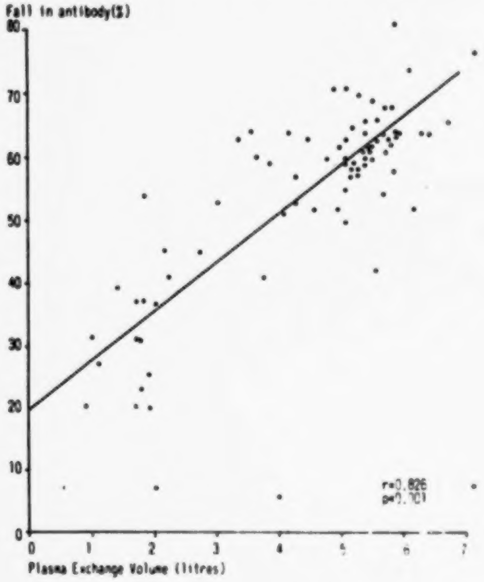
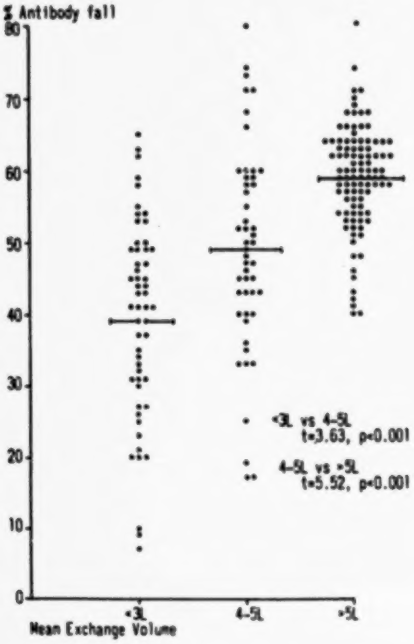
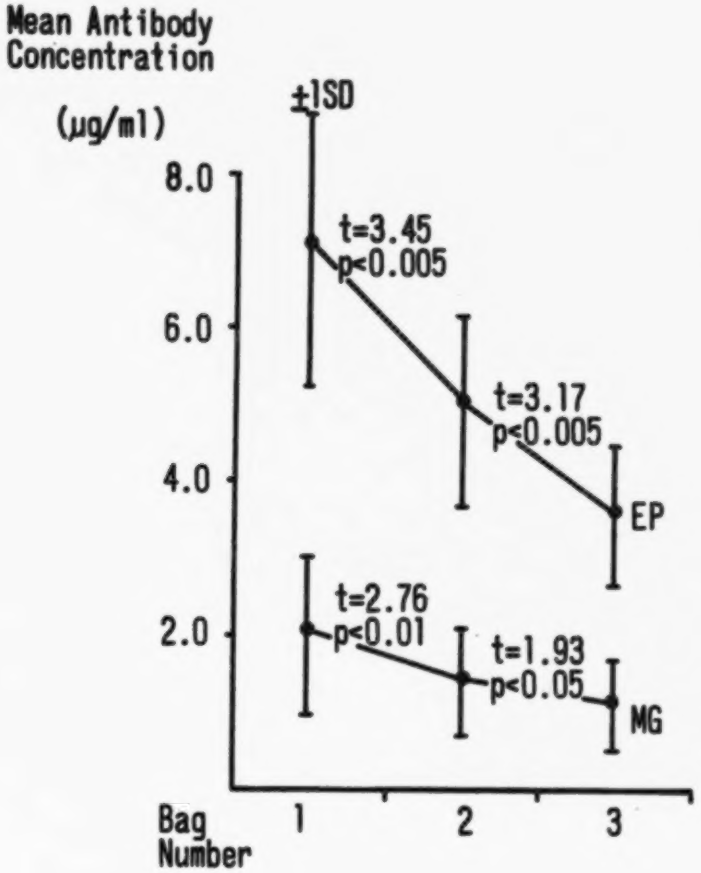


Figure 4.--Mean percentage antibody fall in mothers having mean plasma volume exchanges of <3, 4-5, and >5 liters



(Horizontal bars indicate means)

Figure 5.--Mean antibody concentration (±1SD) in successive collection bags (patients EP and MG)



Finally, we found a direct correlation between the percentage of antibody reduction per run and the daily antibody rise thereafter (figure 6). That suggests either rapid equilibration between antibody-containing tissues or accelerated synthesis, or both. When we compared changes in amniotic fluid OD values and circulating anti-D levels over 4-week periods (figure 7), the rates of antibody removal and fetal hemolysis showed no apparent correlation.

Figure 6.--Rate of rise of antibody level related to percentage reduction following last plasma exchange (regression equation y = 1.99x - 40.7)

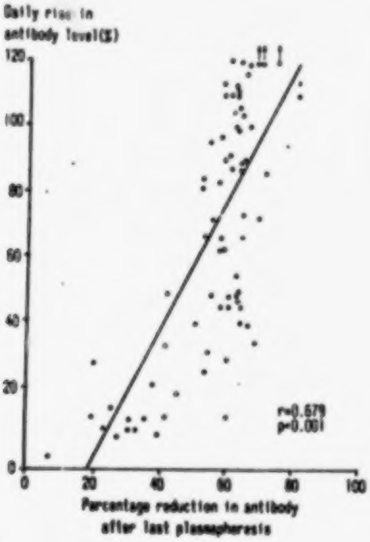
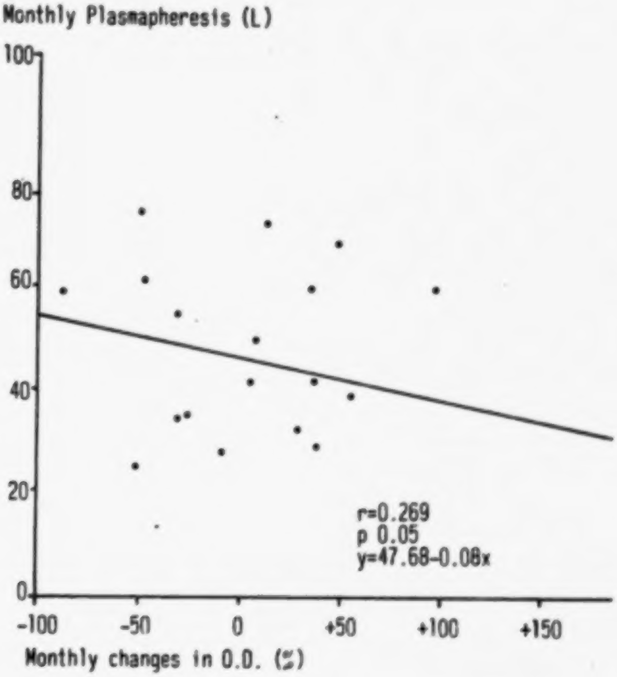


Figure 7.--Monthly changes in amniotic fluid optical density related to monthly volume of plasma exchanged



DISCUSSION

Though prophylaxis with anti-D gamma globulin has greatly lessened the incidence of Rh disease, management of the established condition remains difficult. IUT has never been subject to controlled trials, but results in fetal salvage of less than 50 percent in most series. An exception was the Winnipeg study, which reported 75 percent survival (1). IUT is particularly hazardous before 28 weeks of gestation and, even if successful, is not without hazard to the fetus, from, for example, lymphocyte engraftment (5), trauma, and infection.

Manual plasmapheresis of 1 liter to 4 liters per week (maximum, 36 liters over 6 months) in a recent series achieved a 61 percent success rate when used together with IUT (6). The stage from which the patients were treated was unclear, as was the relative contribution of plasmapheresis and IUT, since amniocentesis changes were not monitored. However, only 3 of 12 pregnancies (25%) treated before 28 weeks were successful. We have, therefore, tried to evaluate the role of intensive and early plasma exchange in the exceptionally severe case.

Outcome of Pregnancy

The major factor influencing outcome is intensity of treatment. Our initial cases achieved poor control with smaller volumes of plasma exchange, having IUT as a deliberate policy. Of the later cases, even exchanges of 20 liters per week failed to control hemolysis in some, while in others, initially unsuccessful, we achieved reversal of a rising amniotic fluid OD value by increasing the weekly volume exchanged.

Effects on Mothers

Great psychological strain was placed on the mothers, both those whose infants died and those whose infants survived. Successes must be weighed against that consideration and against the cost in time and resources involved in long-term treatment. In the United Kingdom, the cost of a 4-liter exchange is about $700, and in the United States it is about $900, excluding staffing costs.

Effects on Rh Antibody and Amniocentesis Values

Our results confirm that methods for predicting severity or monitoring response to therapy of Rh disease are imprecise. Amniotic fluid OD is the most reliable index, yet there is little experience in interpreting results at 25 weeks or earlier. That points up the problems of patient selection and the design of controlled studies. We found amniocentesis essential for monitoring, since it allowed us to intensify or reduce treatment appropriately; circulating antibody levels alone cannot be used as a basis on which to determine treatment changes.

Of particular interest and concern is whether the amount of antibody removed influences its rate of subsequent reaccumulation (3). The mechanisms for primary and secondary antibody responses seem to differ. Rh disease is an example of a secondary or anamnestic response, and the data from Branda et al. (2) make one expect plasma exchange to lower Rh antibody levels without a subsequent rebound. The rate of rebound we observed, however, suggests rapid equilibration with tissue stores, or rapid synthesis, or both. In two cases, an abrupt loss of control occurred in spite of continuing intensive exchange. The lack of overall correlation between the amount of antibody removed and changes in OD values (figure 7) might also make one conclude that even intensive exchange failed to suppress the disease, though the results of individual cases suggest the opposite. The Rh-positive fetus clearly provides a powerful continuing stimulus, and the factors that govern antibody production and fetal hemolysis rates are poorly understood.

CONCLUSIONS

Certain conclusions can be drawn from our study:

1. Severe Rh disease may be treatable from early in pregnancy by intensive plasma exchange alone, but may require IUT in addition.

2. The relationship between rates of antibody removal, its subsequent reaccumulation, and fetal hemolysis remains to be clarified. Even the plasma volume exchangeable with cell separators may fail to control target organ disease.

3. There is no justification for the routine use of less intensive plasma exchange in the management of Rh-affected pregnancies.

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DISCUSSION

Dr. Branda: Thank you very much. This interesting paper is now open for discussion.

Dr. Blumberg: I was wondering-- I have missed somewhere along the line the replacement fluid you were using.

Dr. Graham-Pole: We used freeze-dried plasma or, subsequently, purified protein derivatives, according to availability.

Dr. Blumberg: So you didn't augment, in any rather intensive way, the immunoglobulin levels of these patients.

Dr. Graham-Pole: No, we didn't.

Dr. Blumberg: I must admit, in listening to Dr. Dau's impressive talk, I had sympathetic pains in my buttocks at his description of the 20 cc [of gamma globulin] three times after each exchange; and I wonder if anyone is aware of any evidence that these otherwise normal patients really need this kind of replacement therapy.

I don't know. Maybe it would have an important placebo effect. I would be encouraged to get well, just to avoid further injections.

But seriously, I don't know whether there is any evidence. Certainly, that is a large amount of gamma globulin, probably approaching levels well above what we give our patients who are agammaglobulinemic to prevent infection.

The other point I wanted to make is: I interpret your data on removal and intensifying exchange by increasing volume somewhat differently than you would, I am afraid.

In concurrence with what Dr. Nusbacher had to say about exchange kinetics yesterday, in reference to the data on the first, second, and third bags, and the removal rates, grouping the data by bag tends to make that curve look sharper than it might be. For one thing, even though the results are statistically significant, using a T-test, that curve would become considerably flatter near the end if you grouped the data on a smaller volume basis.

Also, why did you use a T-test to compare two or three numbers there? There were just two patients. If there were more, an analysis of variance and F-statistic would be more conclusive.

Dr. Graham-Pole: There are three patients in whom we looked at all the exchanges.... There are large numbers of data from each patient. We are looking at each run, and you will recall that, in fact, the three subjects we looked at had a total of about 80 runs, something of that sort, so that we are looking at large numbers of data.

We did, of course, monitor normal immunoglobulin levels. When we started to use purified protein derivative, either as well as some freeze-dried plasma or entirely to replace it, we did see temporary falls in overall immunoglobulin values, particularly in...IgM, and all we can say is that these levels recovered quickly, and we didn't see any problem with infection. And we didn't see, certainly, any long-term depletion. We certainly didn't attempt to replace normal immunoglobulin.

Dr. Kennedy: I think you had five patients who had intrauterine deaths, with intensive plasmapheresis, right?

Dr. Graham-Pole: Actually, not quite... [There were] four neonatal deaths.

Dr. Kennedy: Okay. Of these, the delta OD was less than 0.3, right?

Dr. Graham-Pole: Yes; ...in fact, I think in all those cases but one, it was initially less than 0.3. In one case it was higher.

Dr. Kennedy: Was there anything that would indicate that the fetus was getting hydropic during this time? Did you consider intrauterine transfusion in spite of the low delta OD's?

Dr. Graham-Pole: Yes.

Dr. Kennedy: ...is your delta OD perhaps low because you are plasma-exchanging and it is increasing the removal of the bilirubin pigments from the amniotic fluid?

Dr. Graham-Pole: Well, undoubtedly, that is the objective, and I am sure we were achieving it in some of those cases. But of course, four of the intrauterine deaths were...a direct result of intrauterine transfusion. And in only one of those cases was the baby, in a subsequent examination, shown to be hydropic.

In other words, we had...acceptable control of the rhesus disease in the earlier cases, where we...gave relatively nonintensive plasma exchange.

We did have ultrasonography and other information about these infants that I haven't shown data on to try to give us more information about the severity of the disease. Only in one case, as I say, was the baby hydropic in spite of plasma exchange. [The patient] had had previous intrauterine transfusions which resulted in death of the fetus, on two previous occasions.

This isn't one of the patients I showed you. And we were at great pains to avoid intrauterine transfusion, because it was still early in pregnancy and we thought we could probably control the disease.

She broke through at about 30 weeks. The amniocentesis findings went very bad very quickly, in spite of very intensive treatment, and the baby became hydropic and was stillborn at about 31 or 32 weeks.

Dr. Kennedy: Did I understand that you said in only one of the intrauterine deaths was the baby hydropic?

Dr. Graham-Pole: Yes.

Dr. Kennedy: And what was the explanation for the deaths for the others?

Dr. Graham-Pole: Well, in four cases, intrauterine transfusion was the direct cause.

Dr. Kennedy: Oh. Okay.

Dr. Graham-Pole: One of them from premature labor, and three of them from trauma. Yes.

Dr. Kennedy: I misunderstood. I thought those did not have intrauterine transfusion--

Dr. Graham-Pole: No. No, they did; and it was very, very tragic that intrauterine transfusion in our hands resulted in these calamities in spite of relatively good control of the ongoing disease in this very severe group of patients.

Dr. Taylor: I, too, am fascinated by the rather massive immunoglobulin replacement in the myasthenia gravis patients that Dr. Dau presented. I was wondering if he had noticed some adverse clinical experience in these patients suggested by this modality.

Dr. Dau: I thought you were going to ask if we had any adverse reactions to the massive doses of immune serum globulin administered. Other than local pain and discomfort, and the development of allergic reactions in 2 out of the 54 patients, there have not been any adverse reactions to the injections.

The treatment was planned theoretically before we ever did a plasmapheresis in myasthenia gravis and for the reasons I tried to indicate, we wanted to create a semiselective action. We couldn't just take out the autoantibodies, so the next best thing seemed to be to take out all the antibodies and replace the normal antibodies, so that then the cytotoxic drugs administered in conjunction with plasmapheresis, [we hoped] would have a greater action on those cell clones making the autoantibody than on normal lymphoid tissue, because the autoantibody-producing cells would be more stimulated by the plasmapheresis and perhaps more of them would have entered the cell cycle and be dividing where they would be more susceptible to the cytotoxic drugs. That has been shown very conclusively to be the case in animals, I might mention; but it can't be demonstrated in humans.

Dr. Khan: I was going to bring up a point of view contrary to some of the prevailing thinking, just as food for thought and maybe to see some comments on it.

You know, the disparate ends to the use of immunosuppressive drugs in conjunction with the plasma exchanges that we are doing: If one looks at the autoimmune diseases, there is at least some evidence that they may be virally induced, or that viruses play a role in them. If it is so, then antibody really is doing a useful service in that it is trying to eliminate the virus for us. In doing so, it does eliminate some of the tissue which is the host for this viral particle. But at the same time, we have large amounts of immune complexes circulating around, or some antigens which may be interfering with this natural mechanism, which will at least eliminate the disease.

Now, if, in such circumstances, we do plasma exchange, we are removing the complexes and possibly the antigen, and may give the body a chance, or the immune mechanism a chance, at eliminating the disease. It may take a long period of time; but it may be that the results achieved this way will be more curative than palliative.

So we must keep that in mind, and see if it really is justifiable to immunosuppress these patients while we are removing the complexes; at the same time, we are really compromising the immune mechanism, which may have a chance at getting rid of the disease. There are other reasons one could discuss—the reasons why one should avoid immunosuppressive agents. For example, steroids will suppress the clearing mechanism that is the reticuloendothelial function.

So I think...maybe somebody should do a randomized, extremely controlled, very blind study, especially in the neurologic disorders, where there is evidence for such viral implication and where they compare the plasmapheresis versus plasmapheresis and immunosuppressive drugs.

Dr. Graham-Pole: Other investigators have looked at this question in myasthenia gravis, and have found that without adequate immunosuppression--that is to say, steroids have been studied, just the use of steroids without cytotoxic drugs; just...cytotoxic drugs without steroids; or no immune suppression, just plasmapheresis-- Under these conditions, one sees a much more rapid return of autoantibody levels. One sees a poor clinical response, and in almost every case, one sees clinical relapse following the termination of plasmapheresis, in myasthenia gravis.

PLASMAPHERESIS IN THE TREATMENT OF SYSTEMIC LUPUS ERYTHEMATOSUS

John Verrier Jones, B.M., F.R.C.P.
Rush-Presbyterian-St. Luke's Medical Center
Chicago, Illinois

The clinical and pathological features of systemic lupus erythematosus (SLE) are directly related to the deposition in organs and tissues of antigen-antibody complexes (8). In cases of active SLE, immune complexes are also found in the serum, and their level correlates with the activity of the disease (5,12).

The presence of free immune complexes in the circulation is not necessarily followed by permanent tissue damage. Two important protective mechanisms have been described: the clearance of immune complexes from the circulation by cells of the mononuclear phagocytic system (4,10) and the removal of deposited immune complexes from tissues by the scavenging activity of neutrophils (2). In a patient with active SLE, a number of forces are clearly in balance. DNA, RNA, and other nuclear and cytoplasmic antigens are released into the circulation where they initiate antibody production. Circulating antigens and antibodies combine to form immune complexes, some of which will be deposited in tissues, initiating an inflammatory response. Protective mechanisms will lead to inactivation of some of the immune complexes either before or after tissue deposition. The effectiveness of therapeutic measures in SLE can be judged by their ability to lower the level of immune complexes in the circulation and, indirectly, to facilitate normal protective mechanisms. Recent studies have shown that the capacity of the spleen to clear heat-damaged or antibody-coated red blood cells (by implication, an important index of mononuclear phagocytic cell function) is impaired, probably by overload, in patients with active SLE, and that the response to corticosteroid therapy, or to plasmapheresis, may be accompanied by a return of splenic function towards normal (3,9).

In July 1974, we used plasmapheresis for the first time to treat a patient severely ill with SLE. The results were encouraging (6), and we went on to study a series of 14 patients with SLE of varying severity (7). The patients in this group were treated with plasmapheresis 3 or 4 days per week with the object of removing 2 liters of plasma at each session. Replacement was with either fresh-frozen plasma (FFP) or plasma-protein fraction (PPF), from which cryoprecipitable material had been previously removed. Eight of the fourteen patients showed either a clinical improvement or clinical improvement with immunochemical changes. Three patients showed no evidence of a response to plasmapheresis, and three were regarded as unevaluable.

Of the eight patients who responded to plasmapheresis, three showed evidence of high levels of immune complexes, measured by an anticomplementary (complement consumption) assay and by platelet aggregation (6). One patient was deteriorating clinically on 60 mg/day of prednisone and presented with vomiting, edema, proteinuria, and a fever. Her leukocyte count was extremely low (600/mm^3), and platelets were reduced to 80,000/mm^3. Her renal biopsy showed areas of fibrinoid necrosis in all glomeruli, with massive subendothelial electron-dense deposits. Following plasmapheresis, her leukocytes and platelets returned to normal within 2 weeks. Her clinical condition improved remarkably. Joint pain, chest pain, and proteinuria all subsided, and C3 and C4 levels, initially profoundly reduced, began to return to normal.

Immune complexes measured by anticomplementary activity (AC) and platelet aggregation (PA) both showed a steep fall to normal. It was striking that the fall took place during a period when she was not undergoing plasmapheresis. This was one of the observations that led us to postulate that plasmapheresis, by lowering the load of circulating immune complexes, might be effective in patients in whom the mononuclear phagocytic system (reticuloendothelial system) was temporarily saturated, by enabling the system to recover from overload and clear immune complexes normally.

Our second patient (a 17-year-old boy) showed a similar clinical and immunochemical response on his first admission. On a subsequent occasion, when he had developed a characteristic butterfly rash of the face, the rate of plasmapheresis was increased from 2 liters per week to 10 liters per week, and within 2 weeks the rash had disappeared completely.

The third responding patient, who developed cryoglobulinemia and a positive LE cell test 9 years after presenting with a seropositive erosive arthritis, showed clinical improvement with an increase in weight and in exercise tolerance and a decrease in cold sensitivity following plasmapheresis. Her immunochemical abnormalities showed a slow overall improvement after a prolonged period of plasmapheresis.

Five patients had documented evidence of clinical improvement despite immunochemical tests which suggested that immune complexes were either absent or present at low levels. The tests available to us at that time were markedly less sensitive than those we are currently using to detect immune complexes. In four of the five patients, the typical butterfly rash of SLE disappeared within 10 to 14 days of the start of plasmapheresis. In one of the four, plasmapheresis was carried out before treatment with prednisone. In the remaining three, the rash had persisted despite a dose of prednisone that had been constant for at least 1 month. The fifth patient, with no skin rash, showed a rapid resolution of pleurisy and pericarditis when plasmapheresis was commenced.

Three patients showed no response to plasmapheresis. One, a Kenyan of Asian extraction, died with cerebral lupus, despite treatment with 60 mg/day of prednisone and 150 mg/day of azathioprine. Plasmapheresis was carried out at a rate of 5 liters in 5 days, followed by a further 6 liters in 3 days. This had no effect on her clinical condition. She remained stuporose, had a series of convulsions, and died. One patient, with SLE and thrombocytopenia, had suffered a major cerebral infarct and digital gangrene when plasmapheresis was carried out. Her condition continued to deteriorate, and she died. At necropsy she was found to have multiple cerebral infarcts and myocardial fibrosis. The third patient, who showed no improvement after a 5-liter plasmapheresis, responded rapidly to prednisone.

In the three remaining patients, it was not possible to evaluate the effect of plasmapheresis. In one there were problems of venous access, and in one the dose of prednisone was increased at the same time that plasmapheresis was commenced. One patient continued to deteriorate despite the removal of 3 liters of plasma, and prednisone was therefore added to the therapy.

Of the eight patients who responded clinically to plasmapheresis, three who entered the study with high levels of immune complexes showed a strikingly rapid fall of those complexes. In two cases, discussed in detail in (7), the half disappearance time for circulating immune complexes, following plasmapheresis, was 29 hours and 22 hours. The rate of clearance was substantially greater than could be accounted for by the volume of plasma removed. Those results gave support to our hypothesis that in some patients with SLE the mononuclear phagocytic system might be saturated by high levels of circulating immune complexes and that plasmapheresis, by lowering the load, might enable the system to clear itself and effect the removal of additional complexes.

The 14 cases in the Bristol study showed clearly that plasmapheresis had a therapeutic effect in some cases of SLE. It left unanswered several important questions, however. First, we made no attempt to study the duration of the therapeutic effect; second, we needed more sensitive methods for measuring immune complexes in those patients in whom the anticomplementary assay and platelet aggregation were negative.

We have now studied an additional eight patients in Chicago in an attempt to answer those questions. All patients were either stable or deteriorating clinically and immunochemically at the time they were studied. Apart from two patients, who were receiving no drug therapy at the time of study, all had been on a constant dose of drugs for at least 1 month prior to plasmapheresis. Plasmapheresis was carried out as before, using a Haemonetics Model 30 blood-cell separator. The frequency of plasmapheresis was variable, ranging from 2 liters twice weekly to 2 liters five times weekly. In our most recent cases, we have planned to carry out 2-liter plasmapheresis on 5 successive days in each of 2 weeks. Following plasmapheresis, we have studied the patients at frequent intervals to determine the duration of the observed changes in immune complexes and antibodies.

In all patients, we have used two further tests for measuring immune complexes. The first is a Raji-cell radioimmunoassay (11), and the second, a solid-phase Clq-binding assay (11). These methods are considerably more sensitive than the anticomplementary and platelet aggregation assays, and results have been positive in all patients with active SLE under study. In addition, we have measured antibodies to both double-stranded (ds-) and single-stranded (ss-) DNA, using a modification of the Farr assay (11).

In brief, we found in five of the eight patients that plasmapheresis was followed by a reduction in the level of antibodies and immune complexes, followed by rebound to pretreatment levels. In the remaining three patients, there was a sustained fall after plasmapheresis, which was maintained for between 6 months and 2 years.

Of the five patients in whom the response was transient, two were treated with plasmapheresis alone. The case illustrated in figure 1 is that of a black female, aged 24, in whom SLE was diagnosed in 1972. She was treated initially with intravenous methylprednisolone, followed by oral prednisone. In 1974, she developed avascular necrosis of the head of her right femur, and in 1975 had a total hip replacement. In March 1978, her symptoms recurred, and her referring physician, who was anxious to avoid further treatment with corticosteroids, asked that she be considered for plasmapheresis. Six exchanges were carried out. Immune complexes were reduced to undetectable levels after each plasmapheresis, and the amount of rebound between treatments was also reduced. Levels of antibody to ss-DNA were reduced from 75 percent to 30 percent. During the 2-week period of plasmapheresis, her symptoms improved. Within 10 days of terminating plasmapheresis, immune complexes detected by the Raji-cell assay had climbed to levels higher than before treatment. Complexes detected by Clq-binding also rose, though less rapidly. Her symptoms returned in the same period. Twenty days after completing plasmapheresis, she commenced treatment with prednisone, 30 mg/day, with rapid symptomatic improvement. The second case treated with plasmapheresis alone showed similar results.

Six patients were receiving either prednisone alone or prednisone with cytotoxic drugs at the time of plasmapheresis. Three showed a transient response to plasmapheresis, with immune complexes and antibodies returning to pretreatment levels within 10 to 20 days of plasmapheresis. Three patients showed a prolonged response to plasmapheresis, with a fall in circulating immune complexes and in antibodies which has persisted for 6 months, 2 years, and 30 months. All three cases were treated with cyclophosphamide for 1 month after plasmapheresis. A representative patient from that group is shown in figure 2. A 16-year-old black girl, she had a 6-month history of facial rash, polyarthritis, fever, and chest pain, with an increased frequency of epileptic seizures. On admission to the hospital, she had pleural and pericardial effusions, congestive cardiac failure, and a hemolytic anemia with positive Coombs' test. Antinuclear antibodies were homogeneous and peripheral, and her renal biopsy showed a diffuse proliferative glomerulonephritis. Her condition continued to deteriorate over a period of 4 weeks in hospital, during which she was treated with prednisone, 100 mg/day. Fourteen liters of plasma were removed over 2 weeks. Her clinical condition improved dramatically: pleural and pericardial effusions disappeared, and her congestive cardiac failure resolved. Seven days after plasmapheresis terminated, she was given cyclophosphamide, 100 mg/day orally for 1 month. Two years later, she remains free of symptoms on prednisone, 15 mg on alternate days. Immune complexes remain undetectable. Antibodies to ds-DNA are 2 percent (normal) to ss-DNA 33 percent (mildly elevated).

Figure 1.--Serial changes in immune complex assays (Raji assay, N $\leq$ 40 µ g/ml, Clq-binding assay, solid phase, N $\leq$ 10 µg/ml), and DNA-binding assays (ds-DNA: N $\leq$ 5%, ss-DNA: N $\leq$ 15%) in relation to plasmapheresis. Plasmapheresis: at arrow. Two liters of plasma removed. See text for clinical details.

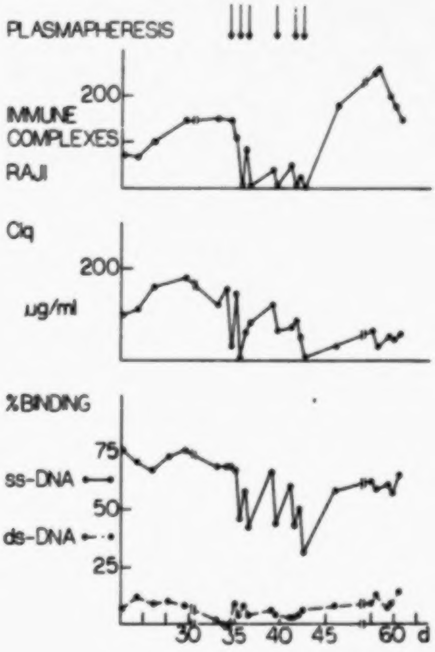
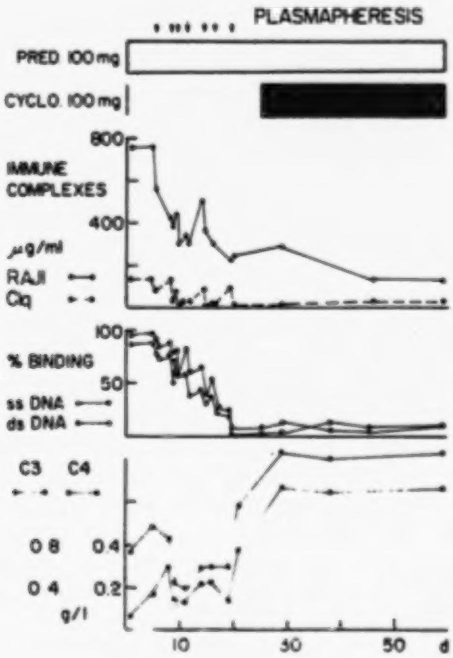


Figure 2.--Serial change in immune complex assays (Raji, Clq-binding) and DNA-binding assays in relation to plasmapheresis. See text for clinical details.



CONCLUSIONS

We have established that in many patients with SLE, plasmapheresis can effectively lower the level of immune complexes and antibodies and may lead to the disappearance of symptoms, including skin rash, arthralgia, pleurisy, and pericarditis. Twelve of twenty-two patients have had documented evidence of improvement. Of the eight patients studied in Chicago, five had a response that was transient, but three have had prolonged remission, apparently attributable to plasmapheresis. A common feature of those three patients is that they were treated with cyclophosphamide for 1 month immediately after plasmapheresis.

We suggest that the rebound in antibody synthesis and immune complex production that immediately follows plasmapheresis may correspond to the synchronization of clones of B-cells, and that during this period they may be particularly susceptible to destruction by cytotoxic drugs. In the next stage of assessing the role of plasmapheresis in the clinical management of SLE, it will be necessary to focus on the most effective drug, or combination of drugs, to prevent the rebound phenomenon and prolong any remission of symptoms that follows plasmapheresis.

In summary, the evidence at present suggests that plasmapheresis may be of therapeutic importance in the management of certain cases of SLE. Those most likely to respond will have high levels of immune complexes and antibodies to DNA and will have failed to respond to conventional drug therapy. It is probable that a cytotoxic drug, such as cyclophosphamide, will be required to block the rebound of antibody synthesis in the period following plasmapheresis.

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DISCUSSION

Voice: Were there any observations with respect to the rate of return?

Dr. Verrier Jones: That really is a critical question. It begins to look as though the rate of return of complexes and antibodies is a little slower in patients who are having azathioprine and prednisone as opposed to those who are not having any other treatment.

I would say that the half-time of return to pretreatment levels, in our cases, is about 4 to 5 days, so that by about 8 to 10 days, they are usually back to where they were initially.

Dr. Goldfinger: I would like to make two comments, one in relation to what was just said. We hear a lot about this concept of rebound, both in terms of immune complexes and in terms of antibody. And this may, I think, throw an unfair light on plasmapheresis as a therapeutic technique.

If we lower the concentration of an antibody by performing plasmapheresis and it returns to its pretreatment level because of normal synthesis, the rate of which has not been changed, you can call it a rebound, if you like, but it seems to have a bad connotation, as though we have worsened the situation. And in many cases, perhaps we have not done that at all. We are just seeing the normal synthetic rate, and of course, the concentration is returned to pretreatment levels.

The second point is concerning the issue of immune complexes. One of the great values of plasmapheresis is that we can often treat a disease and be successful, even though we don't know what the heck we were doing when we got started.

In our studies with rheumatoid arthritis, we thought we knew what we were doing. We were attempting to remove lymphocytes because of previous studies demonstrating that thoracic duct drainage would improve patients; and secondly, we thought we could remove immune complexes, which are thought to have an important role in the joint disease.

What we found was that we got no better results when we removed lymphocytes than when we just removed plasma. We removed plasma and lymphocytes, or just plasma. We saw no difference; all the patients did well.

And secondly, we saw no correlation with immune complexes. We always could lower the immune complex concentration, just as you have, and often decrease these to undetectable levels. However, as we decreased the frequency of apheresis to twice a week, from three times to twice and then to once a week, we found a return to pretreatment levels of immune complexes, and yet the patients remain in remission. And they remain in remission for several months, at least.

And so here we thought we knew what we were doing, but, undoubtedly, immune complexes are not the answer, at least in rheumatoid arthritis.

Dr. Verrier Jones: That's interesting. Thank you for the comments. If I can just add to the first comment: The rebound-- I agree with your comments on it, but another thing that I think is worth emphasizing is that our patients end up having rebounded with the same level of complexes that they did before. And I can't stress too highly that this may be 200 micrograms or 600 micrograms/ml, something characteristic of the patient at that time. And I think we need to know a lot more about why it doesn't go on increasing, or stop at a low level.

The other point on the frequency of the apheresis is certainly important, and our studies would suggest that in lupus, particularly, if you just do it twice a week, then the rebound occurs between ahphereses. If you do it as often as five times a week, then there is a substantial drop in the course of 5 days.

Dr. Grossman: As a hematologist, I would first of all like to thank Dr. Verrier Jones very much for pointing out to me how fortunate I am that most of my patients do not survive the therapy that we offer them. We will certainly return to Vancouver with a new-found enthusiasm for our work, and we will probably discontinue the new protocol that we are in the process of devising.

I just have one question: We have noted a very remarkable improvement in cerebral manifestations of lupus. We have seen patients ranging from just showing mild drowsiness, confusion, lack of interest in their surroundings at the times that we instituted plasmapheresis for other indications, to patients that have been deeply comatose. And in all cases, the cerebral manifestations of what we believe is a vasculitis have improved dramatically with plasmapheresis, over and above what other manifestations, clinical manifestations, in the disease have done. And I wonder if that has been your experience, as well.

Dr. Verrier Jones: Yes, thank you. I showed you the first of the prolonged responders, who was a [patient with] lupus cerebritis who was admitted in a coma in 1976. She improved slowly over 2 weeks, and is now living in Calgary and leading a completely normal life.

Dr. Grossman: If it's possible, in Calgary.

Dr. Verrier Jones: One of the cases we studied in Bristol had lupus cerebritis and failed to respond to apheresis. I think our problem with lupus cerebritis has been that any other physician treating lupus cerebritis will start to raise the dose of steroids as soon as the patient shows mental changes; and it is therefore impossible for us to fulfill the criteria of a patient who is on a stable dose of drugs for a month before treatment.

But your experience is interesting. I have heard it anecdotally from others, and I think it is important.

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LONG-TERM PLASMA EXCHANGE IN SEVERE FAMILIAL HYPERCHOLESTEROLEMIA

Gilbert R. Thompson, M.D., F.R.C.P.
Hammersmith Hospital
London, England

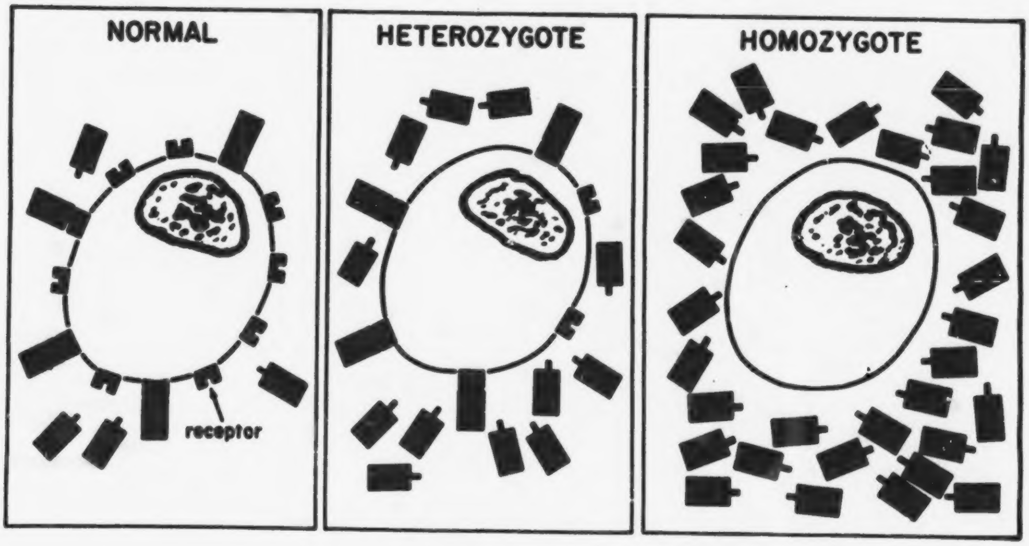
Familial hypercholesterolemia (FH) or familial type II hyperlipoproteinemia is inherited in an autosomal dominant manner. The hypercholesterolemia is due to an excess of beta- or low-density lipoprotein (LDL) in plasma, this being secondary to an underlying defect of LDL catabolism, and is accompanied by progressive accumulation of cholesterol in the skin, tendons, and arterial wall.

METABOLIC BASIS OF FH

The biochemical basis of the metabolic defect in FH has been explored by Brown and Goldstein (4) and is reviewed in detail elsewhere (20). Briefly, those authors showed that cultured fibroblasts from the skin of FH homozygotes lacked the high-affinity LDL-receptors demonstrable in normal fibroblasts and that, as a result, LDL degradation was impaired. Heterozygotes possess about half the normal complement of receptors, and this is reflected in their fibroblasts having an intermediate capability to bind and degrade LDL (8), as illustrated in figure 1.

Although FH fibroblasts oversynthesize cholesterol _in vitro_ (9), there is little evidence of any increase in whole body cholesterol synthesis _in vivo_, as pointed out by Myant (15). However, studies of the turnover of apo-B, the protein moiety of LDL, have confirmed the existence of a catabolic defect in that the fractional catabolic rate (FCR) of LDL-apo-B is about half of normal in heterozygotes and a quarter of normal in homozygotes (21). In addition, there is evidence in homozygotes of oversynthesis of apo-B, some of which is secreted directly into plasma as LDL instead of as very-low-density lipoprotein (VLDL) (16), which is normally the precursor and sole source of LDL. Thus the hypercholesterolemia of FH is largely secondary to abnormalities of the metabolism of apo-B rather than of cholesterol itself.

Figure 1.--Diagrammatic representation of partial and total deficiency of LDL receptors on surface of fibroblasts from subjects with heterozygous and homozygous FH respectively [adapted from Goldstein and Brown (4).]



CLINICAL CONSEQUENCES OF FH

Homozygotes, who occur with frequency of fewer than one per million, present during childhood with cutaneous and tendon xanthomata and extreme elevation of serum cholesterol, to levels of 600 to 1,000 mg/dl. Subsequently, they develop symptoms and signs of atheromatous involvement of the aortic valve, root of the aorta, and coronary arteries. Death is usually sudden, due to acute coronary insufficiency secondary to coronary ostial stenosis, and is almost invariably before the age of 30 (22). At post mortem a striking finding is the localized nature and intensity of the atheromatous infiltration of the aortic valve and root of the aorta. In some instances the aortic valve becomes markedly narrowed by fibrosis (3).

Heterozygotes occur with a frequency of 1 in 500 and often remain undetected until the third or fourth decade when they present with symptoms of atheromatous involvement of coronary or peripheral vessels. Their degree of hypercholesterolemia is less than in homozygotes, usually being between 300 and 500 mg/dl, and their tendon xanthomata appear much later in life. They have a considerably increased risk of premature death from coronary heart disease (CHD); the mean age of death in males ranges from 40 to 55, whereas affected females live about 9 years longer (10).

CONVENTIONAL THERAPY OF FH

All forms of treatment of FH aim at reducing the plasma level of LDL in the hope that this will prevent or reverse the cardiovascular consequences of uncontrolled hypercholesterolemia. The various methods in current use are reviewed in detail elsewhere (22). In brief, however, some reduction in LDL can be achieved by a diet low in saturated fat and cholesterol and high in polyunsaturated fat; supplementation with cholesterol-lowering drugs is usually required also. The drug of choice is cholestyramine, an anion exchange resin which binds bile acids, preventing their reabsorption and promoting conversion of cholesterol into bile acids. The drug effectively lowers LDL levels in most heterozygotes but has little influence on the hypercholesterolemia of homozygotes, who respond better to large doses of nicotinic acid. Such patients pose an especially difficult therapeutic problem. Ileal bypass, which has effects on bile acid metabolism similar to those of cholestyramine, is a highly effective means of reducing the serum cholesterol in heterozygotes (5) but not in homozygotes. Nor has the portacaval shunt lived up to its early promise--further experience with this procedure has shown only a 30 percent reduction in the serum cholesterol level of homozygotes, as opposed to the 70 percent reduction described in the initial report of Starzl et al. (18). It was because of the difficulty of treating homozygotes and their appalling prognosis that we decided to experiment with the use of plasma exchange. Before describing our results, however, it is relevant to briefly review some earlier attempts to control hypercholesterolemia by plasmapheresis.

PLASMAPHERESIS FOR FH

Plasmapheresis is defined as the sequential procedure whereby blood is removed and the red blood cells separated and returned to the donor after resuspension in isotonic saline (19). The term plasma exchange is preferable when describing the use of a cell separator to simultaneously remove plasma and replace it with either fresh-frozen plasma (FFP) or plasma-protein fraction (PPF).

Plasmapheresis was first used to treat a patient with homozygous FH by deGennes et al. (7). The procedure was performed every 2 to 3 days and resulted in a 40 percent decrease in serum cholesterol over a period of 4 months. Apstein et al. (1,2) obtained similar results in another homozygote treated daily for periods of 5 to 9 days; the serum cholesterol returned to its previous level within 10 days of discontinuing treatment. In both instances, the inconvenience of frequent small volume plasmaphereses clearly outweigh the potential benefits of this novel therapeutic approach.

PLASMA EXCHANGE FOR FH

Turnberg et al. (24) were the first to exploit the potential of a cell separator to treat hypercholesterolemia. Over a period of 6 months they successfully relieved the symptoms of xanthomatous neuropathy in a patient with primary biliary cirrhosis by performing 1- to 2-liter plasma exchanges with FFP at 2 to 4 week intervals. Subsequently, Thompson et al. used plasma exchange to treat FH homozygotes, as first reported in 1975 (23). Since then we have carried out over 300 plasma exchanges in a total of 8 patients, 4 homozygotes and 4 heterozygotes, using an Aminco Celltrifuge and heparin as the anticoagulant, as described elsewhere (22).

Initially, we used FFP as the exchange medium but we soon switched to PPF, the constituents of which are shown in table 1. Note the absence of any cholesterol. Because of the low content of calcium and potassium we routinely add 1.5 mEq of K^+ (as potassium chloride) and 0.45 mEq of Ca^{++} (as calcium gluconate) to each 400 ml of PPF. This prevents the hypocalcemia that occurs when unsupplemented PPF is used (6).

Table 1.--Composition of plasma-protein fraction blood products

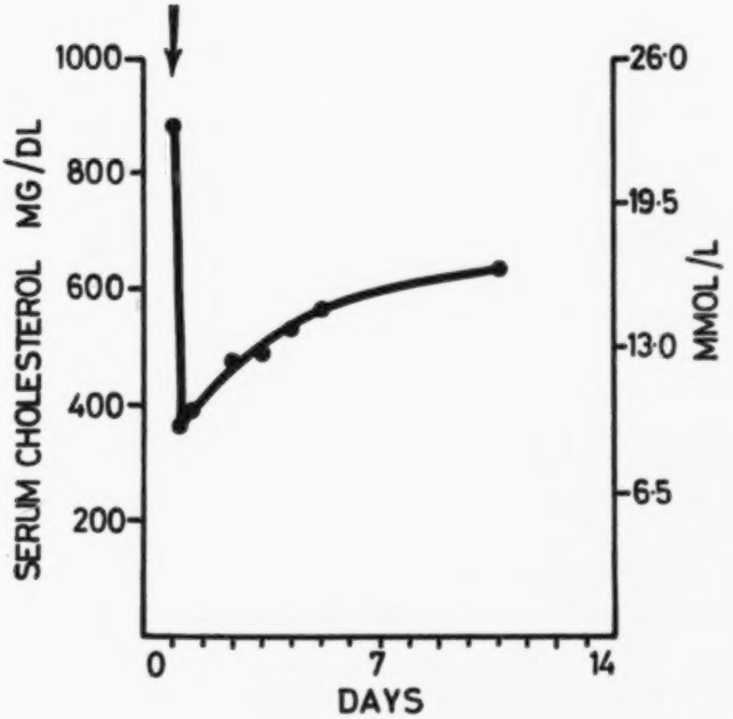
	Specified*	Measured	Normal Plasma
Total protein, g/l	43.0- 47.5	45.0	65.0 - 80.0
Albumin g/l	> 90%	39.0	35.0 - 55.0
Na^+ mmol/l	140.0-160.0	155.0	136.0 - 149.0
K^+ mmol/l	<2.0	1.0	3.8 - 5.2
Ca^{++} mmol/l	-	1.175	2.15 - 2.65
Po_4^{--} mmol/l	-	0.04	0.8 - 1.4
HCO_3^- mmol/l	-	3.0	24.0 - 3.0
Cl^- mmol/l	100.0-200.0	-	100.0 - 110.0
pH	6.7- 7.3	6.75	7.36 - 7.42
n-octoate mmol/l	3.6		
Citrate mmol/l	< 15.0		

* Blood Products Laboratory, Elstree, Herts, England.

Effects on Serum Lipids and Lipoproteins

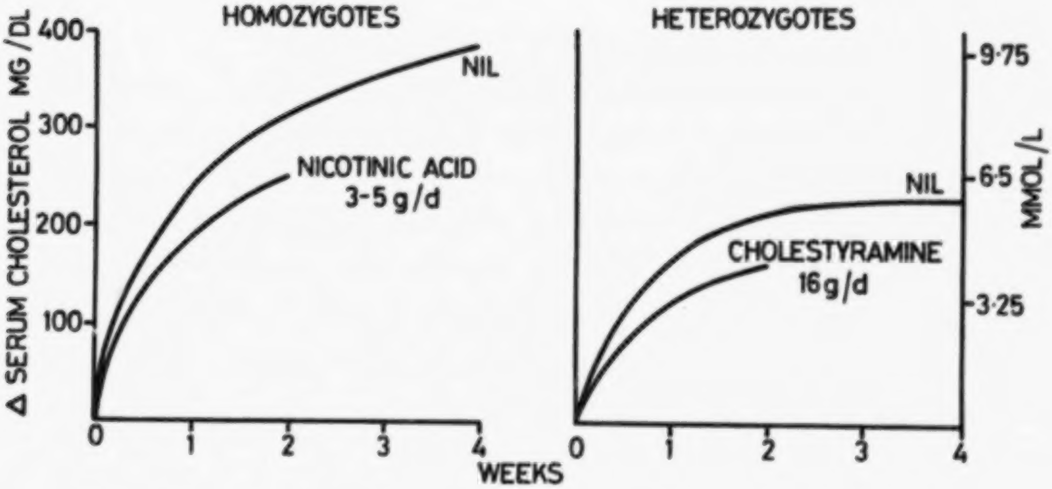
The effect of a single plasma exchange in a previously untreated homozygote is shown in figure 2. The rapid decrease in serum cholesterol during plasma exchange is followed by a slow rebound. The extent of the initial fall depends upon the volume of PPF exchanged and can be calculated from the equation $F = e^{-p}$, where F is the fraction of cholesterol remaining in plasma at the end of the exchange and p is the ratio between the volume of PPF exchanged and the plasma volume. If $p = 1$, then $F = 0.37$; i.e., exchanging the equivalent of one plasma volume of PPF should reduce the serum cholesterol by 63 percent. In actual practice we have observed decreases of 61 percent after 3.2-liter exchanges and 70 percent after 4-liter exchanges. Those values are in good agreement with theoretical estimates and suggest that there is no shift of extravascular cholesterol into the intravascular compartment during the actual exchange, although there is evidence that such a shift does occur subsequently (23).

Figure 2.--Changes in serum cholesterol in a homozygote during and after a 2-liter plasma exchange with PPF (shown by the arrow)



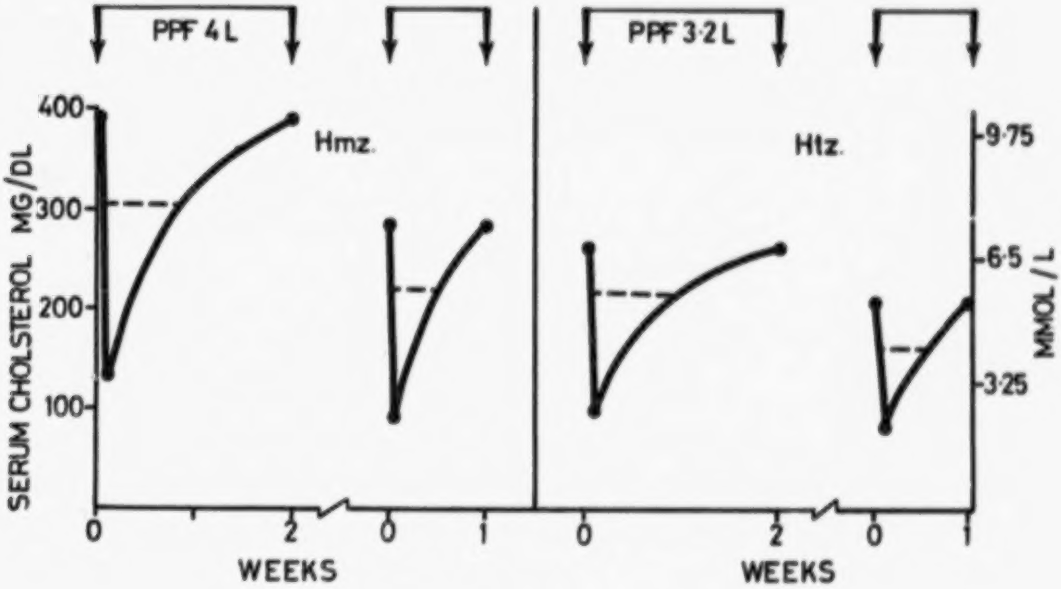
The rebound in serum cholesterol after plasma exchange is greater in homozygotes than in heterozygotes, ranging from 150 to 250 mg/dl during the first week. The rate of rebound can best be retarded by giving nicotinic acid, 3 to 5 g/day, in homozygotes and cholestyramine, 16 g/day, in heterozygotes, as illustrated in figure 3. Studies with labeled LDL-apo-B have shown no increase in synthetic rate following plasma exchange (17), nor is there any immediate change in fecal steroid excretion.

Figure 3.--Comparison of rates of rebound of serum cholesterol from postexchange value in homozygous FH on nil treatment versus nicotinic acid, and in heterozygous FH on nil treatment versus cholestyramine



The effect of the frequency of plasma exchange is illustrated in figure 4. In homozygotes, repeated 4-liter exchanges at weekly intervals resulted in a mean serum cholesterol level of 250 mg/dl, which is similar to that achieved in heterozygotes by 3.2-liter exchanges at fortnightly intervals and is within the normal range for the population at large.

Figure 4.--Effects of repetitive plasma exchanges (shown by arrows) on mean serum cholesterol, calculated by integrating the area beneath the rebound curve. The mean levels (shown by the broken lines) are contrasted in homozygotes (Hmz) and heterozygotes (Htz) undergoing 4-liter and 3.2-liter plasma exchanges respectively, at both 1- and 2-week intervals.



Serum triglycerides decrease markedly after plasma exchange but are back to normal within 24 hours. High density lipoprotein (HDL) cholesterol decreases in parallel with LDL cholesterol but returns to the preexchange level more rapidly (22), presumably because proportionately more HDL than LDL is located in the extravascular space.

Clinical Results

The beneficial effects of plasma exchange can best be exemplified by considering the progress of three patients. A. R. is a 26-year-old female homozygote in whom angiography showed narrowing of the aortic root and a gradient of 34 mm across the aortic valve (23). She was initially treated with plasma exchange at monthly intervals for 1 year but subsequently underwent a portacaval shunt (16). This caused only a transient lowering of her serum cholesterol, which has ranged between 500 and 600 mg/dl during the succeeding 3 years in spite of various hypocholesterolemic drugs. Repeat angiography showed left ventricular hypertrophy and a fourfold increase in the gradient across her aortic valve. Recently she had to undergo an aortic valve replacement and aortocoronary bypass grafting.

D. L. is a 16-year-old homozygote in whom angiography showed a narrowed aortic root and a hemodynamically insignificant aortic gradient of 19 mm. During the past 3 years he has undergone plasma exchange at 1- to 2-week intervals, combined with nicotinic acid, 2 to 3 g/day, and has shown substantial decreases in the size of his cutaneous and tendon xanthomata. There has been no change in the angiographic appearances nor in the aortic gradient. His serum cholesterol averages 250 to 350 mg/dl depending upon the frequency with which he is exchanged. He has undergone an uneventful puberty during this time and remains asymptomatic.

A. S. is a 47-year-old male heterozygote who presented with angina and a serum cholesterol of 350 mg/dl, which decreased to 250 mg/dl on cholestyramine. During the past year he has undergone plasma exchange at weekly intervals, and that has reduced his serum cholesterol still further, to a mean level of less than 200 mg/dl. His angina has improved and angiographic studies have shown a 60 percent increase in the caliber of stricture of the anterior descending branch of his left coronary artery.

These examples indicate that plasma exchange at 1- to 2-week, but not 3- to 4-week, intervals may prevent the progression of aortic lesions in homozygotes and perhaps induce regression of coronary atheroma in heterozygotes. Encouraging results have also been reported by Berger et al. (3).

Side Effects

Reductions in platelet count and coagulation factors are short-lived and have not given rise to any clinical manifestations in patients undergoing weekly plasma exchange. Whole blood viscosity measured at low shear rates is reduced by 20 to 80 percent immediately after an exchange but is almost back to normal within 1 week (12). Serum immunoglobulin levels may decrease in patients undergoing weekly exchanges, but none have exhibited undue susceptibility to infection.

Complications are infrequent and consist mainly of hypotensive episodes at the start or rigors at the conclusion of an exchange. Phlebosclerosis at venepuncture sites probably is the most serious problem encountered so far in patients undergoing plasma exchange on a long-term basis.

CURRENT INDICATIONS FOR PLASMA EXCHANGE IN FH

At the present time we regard plasma exchange at 1- to 2-week intervals, combined with nicotinic acid, as the treatment of choice for homozygous FH. The relatively small numbers of patients involved means that a few major centers should be able to provide the service on a national basis [in England] without placing undue strain on their resources. Currently we are treating four homozygotes by long-term plasma exchange at Hammersmith Hospital. The youngest of those patients had to undergo the preliminary creation of an arteriovenous fistula at the wrist, to provide adequate venous access.

The indications for treating heterozygous FH by plasma exchange are less clear-cut. At present, its use should probably be restricted to treating those few patients whose angina cannot be controlled by beta-blockers and who are unable to undergo aortocoronary bypass surgery for one reason or another. If unequivocal regression of coronary lesions were to be achieved by that means, however, it would undoubtedly widen the scope of plasma exchange as well as provide encouragement to proponents of the lipid hypothesis of atherosclerosis.

FUTURE DEVELOPMENTS

The chief factors limiting wider application of plasma exchange are the cost of the procedure and the availability of PPF. We have been unwilling to use PPF partly because of the greater frequency of allergic reactions and the increased risk of viral hepatitis, and partly because PPF contains considerable amounts of cholesterol. Also, we do not advocate using protein-free replacement fluids in view of the stimulatory effects of hypoalbuminemia on lipoprotein synthesis. The major theoretical drawback to plasma exchange with PPF is the fact that this procedure reduces the level not only of LDL but also of HDL. This is clearly undesirable if one accepts the concept that the latter has antiatherogenic properties. One way of overcoming the problem would be to selectively remove LDL from plasma by affinity chromatography. That approach has been explored by Lupien et al. (14), who have used heparin-agarose beads to remove LDL from whole blood. An alternative approach would be to introduce HDL or an analogue into the circulation at the end of a plasma exchange, in the hope of promoting efflux of cholesterol from the arterial wall. One possible candidate is Lipostabil, which consists of polyunsaturated soybean lecithin vesicles suspended in a solution of deoxycholic acid (11). Experimental evidence suggests that the lecithin vesicles get transformed into HDL-like particles after intravenous administration and are subsequently taken up by the liver, together with the cholesterol which they have accumulated during their brief spell in the circulation.

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DISCUSSION

Dr. Grossman: Dr. Thompson...if the *in vitro* studies are correct, would it not make more sense to replace these patients with plasma that contains a normal low density lipoprotein?

Dr. Thompson: There's nothing much wrong with low density lipoprotein in these patients. It is perfectly normal in terms of its protein. The reason why its lipid composition is a bit abnormal is because it is circulating for a long time. It just picks up more cholesterol, with time. The main objective, or what we are trying to achieve, is a reduction in the concentration of LDL. And we achieve that best by exchanging plasma with a fluid which is depleted in LDL.

Dr. Grossman: The defect, then, is on the receptor site on the cell surface, not on the binding site of the protein.

Dr. Thompson: That's right.

Dr. Grossman: Oh, I see.

Dr. Thompson: I tried to make that point; that it was the absence of specific cell surface receptors that was the cause of the abnormality.

Dr. Kennedy: I notice that you and several others are using physiological amounts of calcium in your replacement solutions. And I just wanted to point out that in resuscitations and in massive transfusions it has been shown that calcium-containing solutions are really not necessary and that, in fact, with massive transfusions, infusions of calcium have been associated with an increased risk of cardiac arrhythmias; and I wondered if that might apply to our situation.

Dr. Thompson: We used to get undoubted symptoms of hypocalcemia and occasionally arrhythmias before we started supplementing the PPF with calcium and potassium. And also, we used to get the occasional episode of hypotension. We think that it's quite unjustifiable to use English PPF without supplementing it with calcium and potassium.

Dr. Kennedy: Well, we also get those symptoms with FFP, but of course, that is because of citrate, not because of the calcium.

Dr. Thompson: Whatever the reason, you can prevent those symptoms by appropriately spiking the bottle.

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HEMOPERFUSION AND TOXIN ADSORPTION IN THE TREATMENT OF POISONINGS

Michael C. Gelfand, M.D.
James F. Winchester, M.B.
Georgetown University Hospital
Washington, D.C.

Accidental or intentional drug or poison intoxication is a serious medical problem in the United States and Europe (23,43). Self-poisoning accounts for approximately 20 percent of all medical emergency admissions to the hospitals in the United Kingdom (36), and in the United States there are about 2 million poisonings with 400 deaths in children each year (9).

Overall mortality of patients reaching a treatment facility is low, usually less than 1 percent of those admitted (7). The contribution to mortality is derived mainly from those patients in grade IV coma with hypnotic or other drug poisoning. Inclusion of patients with trivial poisoning in computation of mortality rates gives a false estimate of mortality in severe poisoning. Indeed, in severe hypnotic coma (grade IV) mortality figures of 5 (48), 8 (25), 30 (35), and 34 percent (2) have been reported. In salicylate poisoning, not usually associated with coma, delay in diagnosis and institution of therapy has been associated with a striking 25-percent mortality compared with a 2-percent mortality when treatment is promptly initiated (1).

In view of these mortality statistics,"conservative medical management" is a term which clearly must encompass more than a wait-and-see attitude. Efforts aimed at active drug removal should be begun at the time of discovery of any significant overdosage. Table 1 reviews the techniques that are available to facilitate active drug removal from the poisoned patient. Active drug removal may be commenced in the conscious patient with gastric lavage or an agent to induce vomiting. It is important to remember that in the unconscious or stuporous patient, the induction of vomiting may be hazardous. An oral adsorbent such as charcoal slurry may be ingested to help prevent passage of the drug or toxin into the circulation from the gastrointestinal tract. As shown in table 2, virtually all the commonly ingested drugs, such as salicylates, acetaminophen, barbiturates, glutethimide, and many others (43), are well adsorbed onto orally ingested charcoal. There is probably no place in modern toxicology therapy for the so-called universal antidote, and activated charcoal of vegetable origin is more appropriate.

Table 1.--Techniques of facilitated drug removal

1. Emesis
2. Oral charcoal
3. Forced alkaline diuresis
4. Peritoneal or hemodialysis
5. Charcoal or resin hemoperfusion

Table 2.--Commonly overdosed drugs adsorbed by charcoal slurry

Glutethimide	Diazepam
Barbiturates	Chlorpromazine
Acetaminophen	Amphetamines
Salicylates	Pentazocine

In the great majority of patients, careful support of respiration, vital signs, and fluid status, and use of appropriate antimicrobial care will result in a successful outcome. A small proportion of patients require a greater degree of care. In some instances, it is difficult to determine which patient is in the high-risk category. We have set the guidelines listed in table 3 to help select those patients who might benefit from facilitated drug removal by means of hemodialysis, peritoneal dialysis, or charcoal hemoperfusion (43).

Table 3.--Criteria for consideration of peritoneal dialysis, hemodialysis, or hemoperfusion in drug overdose

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1. Severe clinical intoxication with abnormal vital signs, such as hypotension despite fluid replacement, apnea, severe hypothermia, or a combination of these findings.
2. Ingestion and probable absorption of a potentially lethal dose. This is best determined after gastric lavage has been completed.
3. A drug concentration in the blood that is in the potentially fatal range.
4. A degree of intoxication that impairs the normal route of excretion of the drug, or an underlying disease that impairs the function of a major metabolic or excretory organ for the particular drug involved.
5. The presence of a significant quantity of a circulating toxin which is metabolized to a more serious noxious substance. The metabolic conversions of methanol to formaldehyde and ethylene glycol to oxalic acid are typical examples.
6. Progressive clinical deterioration while the patient is under careful medical management.
7. Prolonged coma with its attendant hazards, such as aspiration pneumonia, septicemia from infected IV sites, and peripheral neuropathy secondary to pressure ischemia.
8. The presence of an underlying disease, such as chronic bronchitis or emphysema, that would increase the hazards of coma.
9. The development of a significant complication, such as aspiration pneumonia.

Once a decision, based upon those criteria, has been made to utilize peritoneal dialysis or hemodialysis or hemoperfusion to facilitate drug removal, the type of procedure chosen will depend upon the resources available as well as the type of toxin ingested. Peritoneal dialysis and hemodialysis are techniques that are well described and quite widely available. As shown in table 4, dialysis is effective in augmenting removal of a variety of toxins and, in the severely overdosed patient, should be used for well-dialyzable agents. Agents that are highly protein bound, have low aqueous solubility, or are poorly distributed in plasma water are relatively poorly dialyzable. For such agents, the technique of hemoperfusion is more appropriate.

Table 4.--Measured drug clearances (ml/min)

	Peritoneal Dialysis	Hemodialysis	Charcoal Hemoperfusion
Glutethimide	*	24-119	135.4 ± 50.8
Pentobarbital	< 10	35	63 - 80
Secobarbital	< 10	15	30 - 75
Phenobarbital	< 10	22	50 - 72
Ethchlorvynol	18	64	36 - 114
Aspirin	25	100	92 ± 23.6
Acetaminophen	< 3	120	190 - 315
Paraquat	*	0-8.5	109 ± 28.6

* Ineffective, or insufficient data.

In the technique called hemoperfusion, blood from the patient is passed directly over activated charcoal, thereby obviating the requirement for aqueous solubility or passage of the drug through an artificial or natural membrane. In addition, in some instances, the chemical bonding between activated charcoal and drug is strong enough to sequester the drug from plasma-binding proteins.

The process of hemoperfusion was introduced by Muirhead and Reid (27), who investigated the use of mixed ion-exchange resins in animals to remove uremic toxins. Later, Schreiner (34) employed a lactated anion-exchange resin column for two 15-minute periods in a patient with pentobarbital poisoning, without a great deal of drug removal. Both of those early resin studies and others (32) were complicated by pyrogenic reactions, electrolyte disturbances, and hemolysis. Although the complications could be lessened by pretreating the ionic resins or using a mixture of cation/anion-exchange resins (22,28), the early resin hemoperfusion devices were considered too dangerous for widespread clinical use.

In 1964, Yatzidis (45) presented work showing that *in vitro* a column containing 200 g activated-charcoal particles could adsorb from plasma 1.5 g to 2.0 g barbital, phenobarbital, and pentobarbital, and 2.6 g salicylic acid and 2.0 g glutethimide. In 1965, Yatzidis et al. (47) reported the use of charcoal hemoperfusion in two patients with barbiturate poisoning, both of whom recovered consciousness after three and five hemoperfusions, each lasting 1 hour. Transient side effects noted were facial flushing, dyspnea, and burning sensations (45,47). Previous application of hemoperfusion in animals and man had been associated with platelet depletion and reduction of fibrinogen concentrations (45). Those reports stimulated a great deal of interest in the field, but subsequent reports highlighted the occurrence of platelet depletion (20,11) and particle embolization from the surface of the charcoal particles (20). Encapsulation of charcoal with albumin-collodion-coating solutions pioneered by Chang (3) and the use of polymer solutions by others (44,12,37) have largely overcome both problems and improved the safety of this therapeutic modality. Uncoated activated charcoal in a fixed-bed system (21) and modern resin hemoperfusion cartridges (31) are not associated with larger platelet reductions than those that result with coated charcoal, and the platelet loss in many reports is on the order of 30 percent (41). Table 5 shows typical sorbents used in clinical and experimental hemoperfusion devices to date.

Table 5.--Typical sorbents used in hemoperfusion devices for drug intoxication

Activated charcoal	Uncoated granular charcoal (loose bed or fixed bed)
	Coated granular charcoal
	Spherical (petroleum-based charcoal)
	Extruded charcoal
Ion-exchange resins	Amberlite series
	Zeolite 225
Nonionic resins	Polystyrene amberlite series (XAD-2, XAD-4)

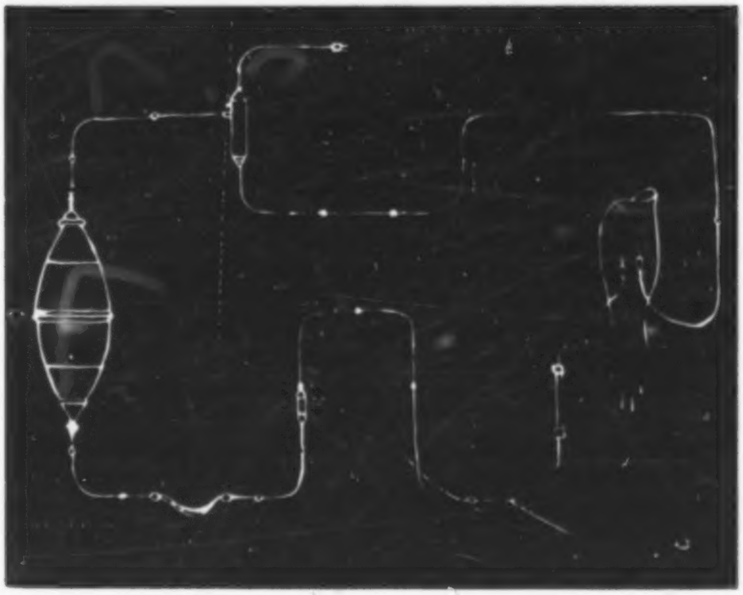
Activated charcoal (carbon) particles prepared from biological sources possess a high surface area for drug adsorption. Adsorption of drugs is dependent on the macropore (500 Åp) and micropore (20 Å) structure, and it is possible to select carbons for substances with a specific molecular weight range. Adsorption seems to accord with the Freundlich isotherm principle that the amount of drug or solute absorbed depends on the initial drug concentration (44,46).

Adsorption profiles of substances demonstrate that the molecular configuration of the compound can determine adsorption (40): urea is poorly adsorbed (45,42); amino acids with branched chain structures are poorly adsorbed; aromatic amino acids are efficiently adsorbed (15,18). Several workers have shown that water- and lipid-soluble substances are adsorbed by charcoal; the molecular weight of those substances varies between 113 and 40,000 daltons (4,39,10). Substances at higher molecular weights are adsorbed less efficiently when charcoal with polymer coating is used (10).

Most clinically available devices use carbon chosen for attrition resistance, washability, and surface smoothness, in addition to pore size, which ensures minimal small particle (fines) release from filled columns (39). Coating techniques also reduce particulate embolization, as mentioned above.

A typical hemoperfusion circuit for treatment of drug intoxication is shown in figure 1. Clinically used sterilized columns generally

Figure 1.--Hemoperfusion circuit using Hemacol ® device



contain 100 to 300 g of activated charcoal for use in cases of poisoning. Each device is flushed with saline prior to clinical use. Blood is withdrawn from the patient via an arteriovenous shunt, or a venovenous shunt, and passed through the hemoperfusion column with a blood pump, in an antigravity direction at rates ranging from 100 ml/min to 300 ml/min. Anticoagulation is achieved with heparin administered intermittently or continuously in sufficient dosage to keep the whole blood clotting time at around 40 minutes.

Available resin cartridges utilize XAD-4, a copolymer of styrene and divinylbenzene that has a special affinity for lipid drugs (31). The preparation and selection of resins are similar to those for charcoal, and particular attention is given to reduction of small particle generation. To date, coating of resins with polymers to improve biocompatibility has not been performed. The technique of activated-charcoal hemoperfusion, pioneered by Yatzidis (45) and refined by Chang (5,6), has found clinical acceptance in the treatment of severe drug overdose. Many centers (19, 38,24,29), including our own (19), have reported on the use of the technique in clinical and experimental drug overdose. Lipid-soluble drugs and protein-bound drugs which are inefficiently removed by hemodialysis can be removed more efficiently by hemoperfusion (5,19). Direct comparative studies of hemodialysis and charcoal hemoperfusion (26) or resin hemoperfusion (33) have shown that hemoperfusion is superior to dialysis in drug clearance. Table 6 shows the wide range of intoxicating substances that have been removed by direct hemoperfusion. Table 7 gives an overview of the efficiency of hemodialysis and hemoperfusion for common poisons; it can be seen that the plasma extraction ratio for most drugs with hemoperfusion is greater than with hemodialysis, while resin hemoperfusion would seem to be more efficient than charcoal hemoperfusion.

Over the last 2-1/2 years we have treated 12 patients with serious intoxication resulting in deep (grade IV) coma. As discussed above, such patients have a very poor prognosis with an expected mortality rate of up to 34 percent in spite of good supportive care. Four of the twelve had taken barbiturates; two, aspirin; four, glutethimide; one, the combination of glutethimide plus aspirin; and one, ethchlorvynol. The 12 patients received a total of 16 hemoperfusions ranging in duration from 1.0 to 7.5 hours. All patients survived. The clinical response of the patients varied depending on the type and quantity of drug taken. Those patients who had ingested barbiturates generally responded promptly during a single hemoperfusion. Patients who had ingested glutethimide generally required more than one hemoperfusion and regained consciousness more slowly and more variably. To date, we have not been required to perform more than two hemoperfusions on any patient with drug overdose.

Table 6.--Drugs removed by hemoperfusion

Barbiturates*

Amobarbital
Butabarbital
Carbromal
Hexabarbital
Pentobarbital
Phenobarbital
Quinalbital
Secobarbital
Thiopental

Nonbarbiturate Hypnotics, Sedatives, and Tranquilizers

Ethchlorvynol*
Glutethimide*
Methyprylon*
Methaqualone*
Diazepam**
Chloral hydrate
Chlopromazine
Promazine
Promethazine
Meprobamate

Analgesics

Acetylsalicylic acid*
Methyl salicylate*
Acetaminophen/paracetamol*

Antimicrobials/Anticancer Agents

Methotrexate*

Plant/Animal Toxins, Herbicides/Insecticides

Aminita phalloides*
Amanitin
Phalloidin
Chlorinated insecticides
Polychlorinated biphenyls
Methyl parathion
Demeton-S-methyl sulfoxide*
Dimethoate*
Nitrostigmine
Paraquat*

Solvents/Gases

Carbon tetrachloride
Ethylene oxide
Trichloroethanol

Cardiovascular Agents

Digoxin*
Procainamide
N-Acetylprocainamide

Alcohols

Ethyl alcohol

Antidepressants

Amitryptiline
Clomipramine
Desipramine

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* Extensively studied *in vivo*.

** Ineffective, or insufficient data.

Table 7.--Plasma extraction ratios for drugs frequently involved in poisoning incidents*

Drug**	Hemodialysis	Charcoal Hemoperfusion	Resin Hemoperfusion
Phenobarbital	0.27	0.5	0.8-0.9
Pentobarbital	---	0.2-0.3	---
Amobarbital	0.26	0.3	0.9
Quinalbital	0.09	0.45	0.8
Carbromal	0.25-0.4	0.5-0.6	1
Glutethimide	0.16	0.6-0.7	0.8
Methaqualone	0.13	0.4-1	0.5-1
Ethchlorvynol	---	0.7	1
Methyprylon	0.4	0.5	---
Tricyclics	0.2-0.5	0.2-0.4	---
Salicylates	0.5	0.5	---
Acetaminophen	0.4	0.5	---
Paraquat	0.5	0.6	0.9
Carbon tetrachloride	---	0.85	---
Trichloroethanol	---	0.4-0.5	---
Digoxin	0.15	0.3-0.6	0.4

* Calculated from data in Winchester et al., Trans. Am. Soc. Artif. Intern. Organs 23:762, 1977.

** Peritoneal dialysis of most drugs < 10 ml/min.

Hemoperfusion is not without its complications. Platelet destruction and removal is the major problem. In the 12 patients, hemoperfusion-induced platelet loss was well tolerated, but, nevertheless, the mean platelet fall was 33 ± 21 percent. Other complications included hypotension (reversed with saline infusion), hypothermia (treated with heating blanket), and mild hypocalcemia in one patient.

Although bleeding is potentially a problem in patients receiving anticoagulants and becoming thrombocytopenic, we did not observe significant bleeding except in the area of the vascular access. Nevertheless, we recommend frequent monitoring of blood platelets during and after hemoperfusion. In patients with low platelet levels, we recommend the infusion of platelets at the termination of hemoperfusion (16).

Transient falls in leucocyte counts were observed, but no significant alterations in hematocrit, hemoglobin, electrolytes, blood urea nitrogen, phosphate, magnesium, alkaline phosphatase, or oxaloacetic transaminase were noted. Small decreases in serum creatinine and uric acid were observed; however, the changes were not statistically significant in that group of patients.

One drug that should be singled out for a brief discussion is acetaminophen. This drug is available in unlimited quantities over the counter, yet as few as 15 to 16 "extra-strength" tablets taken together may result in death from fulminant hepatic failure in 2 to 3 days. Acetaminophen has already become a favorite suicide agent in the United Kingdom (30), where it is known as paracetamol. Because of its ready availability and the severity of its consequences, it is rapidly becoming popular in the United States. This dangerous situation prevails because of the "Tylenol myth," that is, that acetaminophen is safer than aspirin. Perhaps it is less dangerous than aspirin as far as irritating the mucosa of the gastrointestinal tract goes, but acetaminophen may be far more serious when taken in a 10-to-20-pill single dose.

Acetaminophen in the toxic range of 12 g or more results in hepatic necrosis by interrupting the intermediary metabolism of the hepatocyte. The damage has apparently been done if treatment is delayed by 12 to 15 hours or more; therefore, it is highly important to recognize the significance of high acetaminophen dosage and to initiate treatment with extraordinary promptness.

Treatment of acetaminophen poisoning depends on the timing post-ingestion. Within the first 10 to 12 hours after ingestion, treatment is designed to remove the drug, prevent absorption, and prevent interruption of intermediary metabolism of the liver cells. The first (drug removal) is accomplished by inducing emesis or by gastric lavage.

Second, charcoal slurry may be used to bind the drug in the GI tract and prevent absorption. Last, infusion of cystamine or methionine (30,8) may be of benefit in the early stages of intoxication to prevent hepatocyte damage. Hemodialysis, or hemoperfusion, appears to be of little benefit at the early stage although we have treated one patient at that stage with charcoal hemoperfusion. The patient survived, and no liver necrosis occurred. Early use of hemoperfusion remains a single experience and needs further evaluation. The mainstay of treatment is good supportive care. If the ingestion has occurred 1 to 3 days previously, little more can be done than watchful waiting.

In the event of fulminant hepatic necrosis with hepatic encephalopathy, hemoperfusion may be employed. We have treated one such patient who survived, and other reports (13) suggest that hemoperfusion may reverse coma and increase survival from the grade IV hepatic coma of acetaminophen-induced hepatic failure.

The choice of whether to use peritoneal dialysis, hemodialysis, or hemoperfusion, and even the type of hemoperfusion device to use, will largely depend on what is available. As shown here, charcoal or resin hemoprofusion appears to be superior when available, with hemodialysis next best, and peritoneal dialysis least effective (table 4). On the other hand, hemodialysis is superior for the treatment of overdose with aspirin because of the capacity of hemodialysis to correct the accompanying acidosis; hemoperfusion in its present form is not capable of acid-base modification. Methanol intoxication may also be best treated with hemodialysis.

The future of hemoperfusion offers exciting possible advances in a variety of forms of facilitated toxin removal. Charcoal hemoperfusion has been shown to be of benefit in the reversal of such toxic encephalopathies as hepatic coma (14,17). Materials for devices capable of removing specific toxins without removing other substances are being examined experimentally. The addition of a selective adsorbing circuit may soon provide the therapeutic modality for significant breakthroughs in the treatment of metabolic, oncologic, and immunologic disorders.

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DISCUSSION

Dr. Taylor: You made a passing reference to "flypaper" in your columns. I also happen to be a forensic pathologist, and although I haven't looked into the manufacture of flypaper recently, it used to be made with a great deal of arsenic. Have you had any experience with adverse, toxic phenomena that could be attributed to arsenic salts?

Dr. Gelfand: No. I have had little personal experience, but I don't believe that the Becton-Dickinson column has any arsenic in the paper that they use. I will stop using the word "flypaper" as of now.

Dr. Gilcher: Question: Have you thought about, instead of running whole blood over the column, just running plasma over the column and therefore preserving the platelets, using your cell separator to give you the cellular versus the noncellular elements?

Dr. Gelfand: Yes. That represents what we hope will be the evolution. Right now, the Aminco Celltrifuge is not capable of keeping up with the blood flow rates that we think are needed in this procedure, which is why we haven't done it with that.

Others are working with very interesting high-blood-flow separators that may eventually be useful, and may alleviate the platelet problem, indeed.

Dr. Sam Sofer: We're working with that cell separator, too. I have one comment to make: Sometimes people go from dialysis into perfusion over charcoal because the molecules are large enough that they wouldn't be rapidly diffusing through the membrane. However, one word of warning I have is: We found that with very large molecular weight compounds, such as endotoxin, bacterial endotoxin, for example, there is a resistance to mass transfer within the charcoal bead; and you must go to finer particles. Some of the particles that I have seen, on the order of 1 mm or so diameter, will not do the job. And if you need to go to finer particles, it is best that you separate the cells. This is what we have been finding out.

Dr. Gelfand: I think that is a very good point.

Dr. Lauterburg: At our institution, tricyclics are the most frequent cause of drug intoxication. I wonder whether you have had any experience with tricyclics.

Dr. Gelfand: I don't have it on the slide, but we do have some experience. And they are beautifully removed, but as you know, the volume of distribution is quite large. Although the tricyclus are cleared at about 60 to 80 ml/min with a 0.5 to 0.8 extraction ratio, there is only a very small amount of the total body pool of tricyclic available in the plasma at any given time. In the serious, really serious, problems, we will do very extended hemoperfusion or multiple hemoperfusions in order to try to cope with the tricyclic as it comes out of the tissue pool.

Dr. Lauterburg: And how would you assess the clinical benefits?

Dr. Gelfand: I can't yet. We have too few cases. We can remove tricyclics, but it's too early to tell if removal of circulating tricyclic compounds is of benefit.

Dr. Dau: What are the potential hazards of doing repeated hemoperfusion?

Dr. Gelfand: The potential hazard [is] destruction of platelets. With repeated hemoperfusions, the effect on platelets is a continuous one, and platelet levels can drop into very hazardous ranges.

A second problem is the removal of clotting factors. That can be prevented by the infusion of fresh frozen plasma at the end of the procedures, and that is the way we do it in patients with hepatic failure, in whom we have to perform multiple hemoperfusions. Calcium and magnesium can also be removed. Some hypoglycemia and hypothermia can be seen; but that is basically it.

I didn't mean to imply that the problems are insurmountable. These problems are really quite simple to deal with if hemoperfusion is going to be done only once or twice.

Voice: You mentioned, with regard to one drug, the widespread distribution, implying, of course, that it is beyond the intravascular compartment, and you also show the formula by which you calculate removal. Now, in some of the earlier papers, each person who presented a paper had a somewhat different way of presenting either the percent removal of the tumor burden, or the removal of particular plasma compound. And I noticed there were at least three or four different ways in which each investigator has presented that.

Have you attempted to tackle the problem of estimating how much of the toxin you have removed, widespread, not just from the intravascular compartment?

Dr. Gelfand: We have attempted to look into the issue. Of course, we can measure the plasma concentration of the drug and the extraction ratio, thereby estimating removal. It should be noted, however, that one must take into consideration intra-erythrocyte drug in making this determination by rapid separation of determining whole blood concentrations of the particular drug. The charcoal column unfortunately does not lend itself readily to the elution of drug off the column and, thereby, measurement of the amount of drug bound.

Dr. Ray: During this hemoperfusion, you have mentioned, there is reduction of platelet counts. What about red blood cell and white blood cell counts?

Dr. Gelfand: No. There is no significant change in erythrocyte or leukocyte counts.

Dr. Ray: ...How big a molecule can be adsorbed by this procedure? Is there any idea about the molecular size or molecular weight?

Dr. Gelfand: About 40,000 daltons is the upper limit of this particular device....

Dr. Ray: Is there any way that the protein molecules can also be adsorbed?

Dr. Gelfand: Yes, protein molecules are actually adsorbed out; in fact, albumin coating of the column is nothing more than exposing it to albumin.

Dr. Ray: Yesterday and today, in the morning session, various people presented data where an autoantibody poses various kinds of problems in a variety of disease processes. [Can] the hemoperfusion as such, as a single technique, be used effectively for the removal of those autoantibody molecules? Have you tried it in any kind of situation like SLE or other autoantibody-directed disorders?

Dr. Gelfand: No; we have not. It is not, to my knowledge, particularly effective for immunoglobulin removal. We have looked at overall gamma globulin levels, and these are not affected. There may be some selected fraction of gamma globulin which might be slightly reduced; however, overall gamma globulin levels are not significantly altered.

An effective column needs to be more selective. We don't believe that there is any significant role at the moment for standard charcoal hemoperfusion unless specific factors are put on the column, such as coating it with antigen for selective removal of the antibody prior to its use.

Dr. Ray: I have one last question: [Is there] a probability of saturating the charcoal with the particular toxin that you are going to adsorb... and how do you know that it is getting saturated, and whether the patient is going to be done once again with another filter; how [do] you tackle this problem?

Dr. Gelfand: That is a good point.... Various columns are different; and saturations occur more or less rapidly with various columns. For example, the Becton-Dickinson device needs to be changed at approximately 2 hours into the procedure.

I did show our results [on a slide] that throughout the 4-hour hemoperfusion, there is no significant change in the clearance of the various drugs.

Dr. Menitove: Just to follow up on that last question, have you looked at white cells during the procedure? Do they go down, and then shoot back up?

Dr. Gelfand: Well, we have looked at them throughout, and it's not like hemodialysis, where you see the rapid, whatever the mechanism is, pulmonary leukostasis, or--

Dr. Menitove: Yes; looking at [activation of] complement?

Dr. Gelfand: Yes. It doesn't appear to reduce white cell levels in that way.

Dr. Menitove: You have looked at that.

Dr. Gelfand: Yes.

CHARCOAL AFFINITY COLUMN PLASMA PERFUSION FOR THE TREATMENT OF INTRACTABLE PRURITUS OF CHOLESTASIS

Bernard H. Lauterburg
Howard F. Taswell
Alvaro A. Pineda
E. Rolland Dickson
Edwin A. Burgstaler
Gerald L. Carlson
Mayo Clinic and Mayo Foundation
Rochester, Minleosta

ABSTRACT

Removal of protein-bound cholephilic substances such as bile acids by sorbent perfusion might improve the quality of life of patients with severe inoperable cholestasis where the accumulation of bile acids is thought to be responsible for the often distressing and incapacitating pruritus. Plasmapheresis followed by plasmaperfusion with USP charcoal coated onto glass beads was found to be a very efficient and safe technique to remove protein-bound cholephilic anions *in vitro*. Eight patients with intractable pruritus associated with cholestasis secondary to primary biliary cirrhosis (5), Alagille's syndrome (1), or benign recurrent cholestasis (2) were treated with plasmaperfusion with USP charcoal-coated glass beads on 2 to 5 occasions (total of 22). The procedures were well tolerated and resulted in the removal of 126 to 679 µmol of bile acids per procedure following perfusion of 957 ml to 6,100 ml plasma. Plasma concentrations of bile acids decreased 27 ± 13 percent, and the bilirubin levels dropped by 36 ± 7 percent. The difference in the calculated amounts of bile acids circulating in the plasma pre- and postperfusion accounted for less than 20 percent of the calculated amounts of bile acids retained by the columns, suggesting that substantial amounts of bile acids were mobilized from tissue stores. The charcoal-coated glass bead columns retained an average of 81 percent of the bile acids in the perfused plasma without producing significant changes in electrolytes, glucose, and plasma proteins, and without any of the hematologic side effects frequently associated with hemoperfusion. There was, however, a striking relief from itching, which persisted for 24 hours to 5 months following plasmaperfusion. The results lend support to the concept of selective removal by affinity column of a target plasma component whose presence is associated with a disease process. This form of selective removal provides a more efficient and less costly alternative to total plasma exchange.

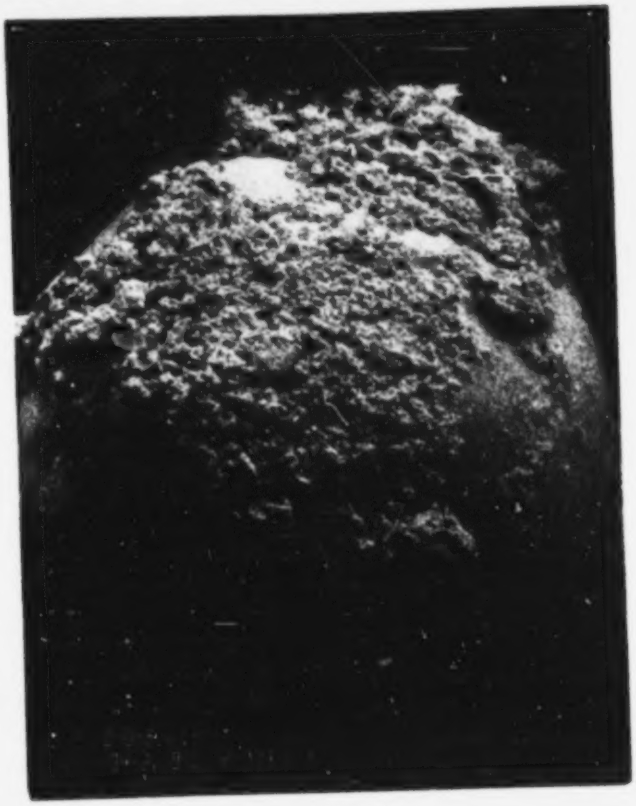
The most distressing symptom of severe, inoperable cholestasis is intense pruritus (1). In many patients, the itching can be relieved with phenobarbital and cholestyramine. However, a number of patients will be virtually disabled by their pruritus despite adequate medical treatment. Bile acids, as well as other substances, accumulate in patients with impaired hepatic excretory function. This occurs despite a striking increase in the renal excretion of bile acids (2). A number of recent, albeit controversial, observations suggest that elevated bile acid levels in the skin may be responsible for the pruritus (2,3,4). With liver and kidneys failing to excrete adequate amounts of bile acids, it is therefore conceivable that removal of bile acids by sorbent perfusion might alleviate the intractable pruritus and thus markedly improve the quality of life of the patients. Unfortunately, neither hemodialysis nor hemoperfusion of currently available sorbents accomplishes a substantial depletion of bile acids (6). In addition, hemoperfusion has invariably been associated with marked losses of platelets and leukocytes, which may become clinically important in patients with liver disease and compromised hemostasis. The hematologic side effects might be prevented if plasma alone interacted with a sorbent. Moreover, plasma perfusion of sorbents might allow the use of novel substances that would be more efficient bile acid adsorbers and that would not have to be biocompatible with formed blood elements. However, because the plasma flow rates generated by cell separators are relatively low, a sorbent with very high extraction rates is required to attain a clinically effective clearance of selected substances.

In an attempt to remove bile acids from patients with severe, inoperable cholestasis and intractable pruritus, we have developed a novel charcoal preparation suitable for plasma perfusion, which very effectively removes bile acids from human plasma and which has promising clinical applications (7,8,9,10).

IN VITRO STUDIES

In a series of *in vitro* experiments where small glass columns containing 6 ml of a sorbent were perfused with human plasma containing 38 nmol per ml of labeled chenodeoxycholic acid and 38 nmol per ml labeled cholic acid, the retention of bile acids by various sorbents was investigated. USP charcoal coated onto glass beads to facilitate perfusion (figure 1) extracted a far higher fraction of the perfused bile acids than various charcoal preparations of larger particle size, anion-exchange resins (Dowex 1X1, Mesh 60 to 140, Dowex 1X2, Mesh 80 to 200), the uncharged resin XAD-2 (Mesh 20 to 50), and albumin coupled to agarose beads. The extraction of chenodeoxycholic acid by the coated glass beads exceeded 90 percent up to a load of 0.15 nmol per ml of sorbent. Cholic acid and cholylglycine were even more efficiently extracted, whereas the extraction of the monohydroxy bile acid, lithocholic acid, was somewhat less. In comparison, the extraction of bile acids by a commercially available charcoal cartridge, immobilized albumin, and XAD-2 was less than 10 percent after an identical load per ml sorbent. The calculated capacity of charcoal-coated glass beads was 20.8 μmol chenodeoxycholic acid per ml sorbent compared to less than 0.5 μmol pet ml for the other sorbents. When uremic human plasma was perfused, the charcoal-coated glass beads almost quantitatively removed uric acid and creatinine whereas urea and most electrolytes were not retained. Only calcium and glucose were adsorbed initially; their removal, however, could be precluded by prior priming of the columns with calcium chloride and glucose. In addition, a substantial fraction of perfused unconjugated bilirubin was retained by the columns [unpublished data].

Figure 1.--USP charcoal-coated glass bead. The diameter of the glass bead is approximately 200 (scanning electron microscopy courtesy J. C. Lewis, Ph.D.)



ANIMAL STUDIES

Subsequent studies in five dogs with experimental biliary obstruction confirmed the efficacy of plasma perfusion of charcoal-coated glass beads *in vivo*. Perfusion of 2.2 times the animal's estimated plasma volume through a column containing 400 ml of the charcoal preparation reduced the plasma bile acid concentration by 40.2 percent and the bilirubin level by 48.2 percent [unpublished data].

STUDIES IN PATIENTS WITH CHOLESTASIS AND INTRACTABLE PRURITUS

After the demonstration that charcoal plasma perfusion was effective *in vivo* and that the procedure was tolerated without serious adverse side effects, we applied the technique to patients with inoperable cholestasis and intractable pruritus. So far, we have performed 22 charcoal plasma perfusions in 8 patients with cholestasis. Five women had primary biliary cirrhosis, two patients had benign recurrent intrahepatic cholestasis, and a 16-year-old girl had Alagille's syndrome, a congenital abnormality of the intrahepatic bile ducts. All patients had intractable pruritus that had not responded to the administration of the bile-acid-binding resin cholestyramine and phenobarbital. Because of their itching, they had been unable to sleep for variable periods of time, and they could hardly tolerate any clothes on their sensitive skin, which was covered with excoriations from their constant scratching. Each patient underwent two to three perfusions lasting approximately 4 hours on consecutive days. Two patients have returned for additional treatment because they were so satisfied with the result of the first set of perfusions. Plasma was obtained with a Haemonetics Model 30 cell separator. Between 957 ml and 6,100 ml of plasma were perfused through a column containing 600 ml of USP charcoal-coated glass beads. Midway through the procedure, the plasma flow was switched to a fresh, identical column in order to maintain a high extraction of bile acids. The column effluent was recombined with the cellular elements and was reinfused into the patient (figure 2). Periodically, the column in- and outflows were assayed for bile acids with a fluorometric method (Sterognost-3 Alpha, Nyegaard and Company, Oslo) or gas liquid chromatography to determine the load of bile acids offered to the columns and the amount of bile acids removed by the columns. Figure 3 shows data from two consecutive plasma perfusions in a patient with primary biliary cirrhosis who happened to have had her chenodeoxycholic acid pool labeled for determination of bile acid kinetics in cholestasis (7). Since the specific activity of the chenodeoxycholic acid did not change during the studies, the areas between the in- and outflow bile acid concentration curves correspond to the amount of chenodeoxycholic acid removed by the procedure. In this particular patient, 254.5 μmol and 239.4 μmol chenodeoxycholic acid were removed, corresponding to 35.7 percent and 33.6 percent, respectively, of the estimated chenodeoxycholic acid pool. The chenodeoxycholic acid removed during each perfusion exceeded the estimated

Figure 2.--Plasmaperfusion of USP charcoal-coated glass beads. Plasma was obtained with a Haemonetic Model 30 cell separator and perfused through a column containing 600 ml of charcoal-coated glass beads. Prior to the perfusion, the sorbent had been heat-sterilized and primed with 5 percent glucose and 10 mg per dl calcium (as $CaCl_2$) to prevent a drop in the plasma concentration of glucose and calcium. The column outflow was passed through a filter of 1.2μ pore size and, after recombination with the formed blood elements, reinfused into the patient.

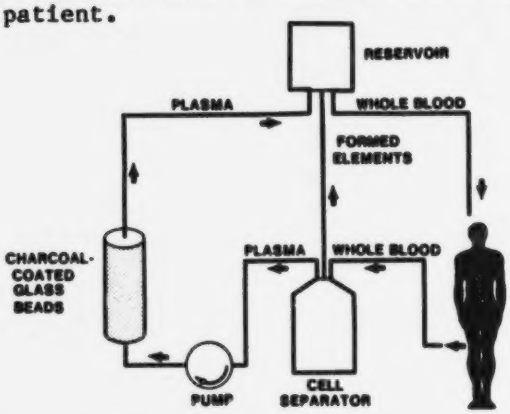
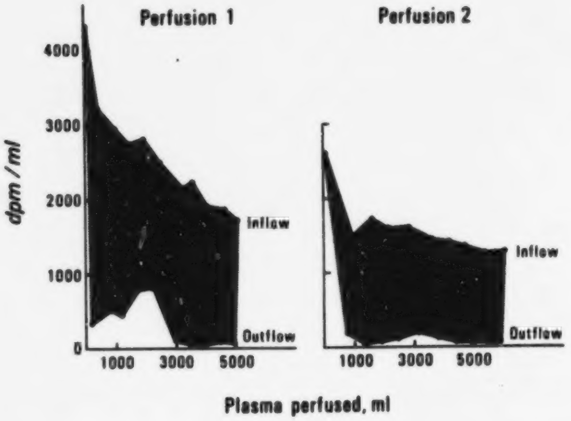


Figure 3.--Bile acid concentrations in column in- and outflow during two consecutive charcoal plasmaperfusions of a patient with primary biliary cirrhosis who had her chenodeoxycholic acid pool labeled with ^{14}C-chenodeoxycholic acid. Since the specific activity of the chenodeoxycholic acid did not change during the procedure and since the extraction of different bile acids by the charcoal preparation is similar, the areas between the upper and lower curves correspond to the amount of bile acids retained by the columns. Arrows indicate time at which plasma flow was switched from first to second of two columns used in each perfusion in order to maintain a high bile acid extraction.



daily input of chenodeoxycholic acid by a factor of 13. Since the extraction of different bile acids is similar *in vitro*, it may be assumed that a total of approximately 500μmol of bile acids have been removed during each of the two perfusions. In the course of the 22 charcoal plasma perfusions, between 126 μmol and 679 μmol bile acids have been removed. The average extraction of bile acids was 81 percent during the perfusions, suggesting that the bile-acid-binding capacity of the columns was far from being saturated. Analysis of the data showed that the removal of bile acids depended on the initial bile acid concentration and the amount of plasma perfused, i.e., the load of bile acids retained by the columns with the estimated reduction in the intravascular pool of bile acids accomplished by the plasmaperfusion indicated that a substantial fraction of the retained bile acids must have been mobilized from extravascular compartments. That hypothesis is supported by the observation of a 65 percent drop in the skin bile acid concentration in the one patient in whom skin bile acid levels were determined.

The plasma perfusions were well tolerated and were not associated with serious side effects. The intensity of the pruritus, as assessed by an independent clinician, decreased in each patient by at least 75 percent following two perfusions. The duration of the improvement, however, was highly variable. In one patient, the pruritus reached the preperfusion intensity 24 hours after the last perfusion, while in the girl with Alagille's syndrome, the itching gradually returned 5 months after treatment. The median duration of substantial improvement of pruritus was 6 weeks.

In view of the subjective nature of the treated symptom, an objective assessment of the clinical value of charcoal plasma perfusion is difficult. Our initial experience, however, suggests that the procedure may result in prolonged symptomatic improvement in some patients. At present, unfortunately, we do not have any criteria for predicting which patients will benefit from the procedure. Extensive studies in one patient have shown that symptomatic improvement may persist after bile acid levels have returned to their preperfusion levels (7). It is, therefore, conceivable that an unidentified, pruritogenic substance other than bile acids has been removed by the plasma perfusions.

CONCLUSIONS

Substantial removal of tightly protein-bound anions such as bile acids has not been possible with hemodialysis or sorbents used for hemoperfusion. The combination of cell separation and plasma perfusion, however, enabled us to select a sorbent that very effectively removes bile acids from human plasma and has a huge capacity to adsorb bile acids. Our studies indicate that sorbent plasma perfusion may have advantages over hemoperfusion in certain clinical situations. The technique may thus serve as a model approach for the selective removal of putative pathogenic substances that are not effectively cleared by hemodialysis or hemoperfusion.

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DISCUSSION

Dr. Taswell: Do we have any comments or questions for Dr. Lauterburg?

Voice: The anticoagulant you indicated you were using was a mixture of ACD and heparin. To what degree, and why?

Dr. Lauterburg: Yes. In our early experience, we just used the ACD, which is suggested by the manufacturer, and which is used in the usual plasmapheresis procedure. However, that did not prevent clotting within the column.

So based on that, and based on some dog experiments showing that one could not increase the amount of citrate because of CNS symptoms, we added heparin. We added 5,000 units of heparin to each bag of the citrate.

Dr. Thompson: Do you think the pruritus correlates with tissue, in other words, skin levels, of bile acids? And have you measured them; and if so, do you think that the amount of improvement that you get reflects a decrease in skin bile acid levels, rather than its noncorrelation with blood levels?

Dr. Lauterburg: Well, as you may know, the literature is somewhat confusing on that. There are some studies which have clearly shown that the bile acid levels in patients who itch are higher than in the patients who do not itch. But not everybody has been able to confirm that.

I also must point out that methodologically, it is rather difficult [to reliably quantitate skin levels of bile acids]. We have tried it, and in the patients I have shown you, the one I showed you last, we measured bile acid levels by extracting the bile acids with a swab dipped into an organic solvent.... There was about a 30 percent reduction in the skin bile acid levels. However, the methodology is somewhat questionable, and I am not sure whether the measured levels are a true reflection of the skin bile acid concentration.

Dr. Thompson: Just one other brief question: Patients with primary biliary cirrhosis very often have extreme hypercholesterolemia. Did you by any chance measure their cholesterol levels in your system?

Dr. Lauterburg: No, we have not. We measured removal of cholesterol in the *in vitro* studies. The cholesterol levels were normal in the plasma we perfused, *in vitro*, and we have not seen any reduction. But we have not measured it in the PBC patients.

Dr. Zuelzer: I was a little surprised, Dr. Lauterburg, and I wonder if I understood you correctly in your comments about bilirubin. Many years ago, we did some comparable studies on neonates, with exchange transfusion for hyperbilirubinemia, and we observed very similar findings to what you described; namely, that in the course of the exchange, you remove more bilirubin than is in the plasma initially.

Did you mean, then, that the difference is a relative one, between bilirubin and bile salts in the efficiency of the procedure? If the bilirubin does pass through the [column]--

Dr. Lauterburg: There is no doubt that bilirubin exchanges with extravascular compartments. There are a number of studies and some computer analyses which demonstrate this.... The bilirubin exchange, at least as suggested by our data, is not as rapid as it is for the bile acids.... But there is no doubt that you can mobilize bilirubin out of tissue. It just is a slower process than the mobilization of bile acids.

Dr. Ray: When you are coating the glass beads with charcoal, after absorption, did you check whether glass beads can pass through the system, along with the blood cells and the absorbed plasma? If glass beads can pass to the system, [it could] produce silicosis kind of symptoms. Did you check this point?

Dr. Lauterburg: Well, there is no way the glass beads can escape. First they are much larger than the charcoal particles--their average diameter is about 100 microns. Then we have a 1.2-micron pore size filter where the plasma passes through, and this filter does not let any charcoal or glass beads pass through.

Dr. Ray: So that filter is kept after the charcoal filter.

Dr. Lauterburg: Yes, the column effluent passes through the filter, so we are not concerned at all about any charcoal...escaping back into the patient. This, by the way, is an advantage over hemoperfusion, where, because of the red cells, you are limited in how far down you can filter the blood that flows from the hemoperfusion cartridge.

Voice: Another question about your comment about the...lack of correlation between the bile acid plasma levels and the presence or absence of itching. You suggested the possibility of another pruritogenic substance;but obviously, the other logical thing to consider is that while the bile acids may return to a high level in plasma, the skin levels are still depleted because of the large amount you have taken out of the extravascular compartment.

Dr. Lauterburg: Yes; but if the bile acids are so quick in coming back into the intravascular compartment, they should be as fast in getting back into the skin. If they equilibrate so fast, as soon as the serum bile acid levels are back to what they were before, most likely the tissue concentrations are going to be what they were before, too.

Voice: Of course, the reasons for them to go from skin to blood are very different than for them to go back from blood into skin. You have a very acute situation, in which you are plasmapheresing and perfusing over charcoal, and you have got a strong gradient; you are pulling them out, whereas when the plasma levels go back up to relatively normal levels, you may not have the same "head of pressure," so to speak, to push them back out into the skin.

Dr. Lauterburg: Although I do not have any experimental data to demonstrate this, the equilibration between intravascular and extravascular compartments should follow first-order kinetics. Thus, the rate of equilibration should not depend on the concentration gradient.

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USE OF BIOLOGICAL IMMUNOABSORBENTS IN THE TREATMENT OF TUMOR HOST

S. C. Bansal
B. R. Bansal
H. O. Sjogren
The Wallenberg Laboratory, University of Lund
Lund, Sweden

B. Husberg
C. Lindstrom
G. Nylander
Departments of Surgery, Pathology and Radiology
Malmo General Hospital
Malmo, Sweden

R. Mark
Department of Pathology, The Medical College of Pennsylvania
Philadelphia, Pennsylvania

ABSTRACT

Protein A, a molecule present on the walls of certain strains of *S. aureus*, has the biological property of interacting and binding to the Fc portion of mammalian IgG and its complexes. Further, *S. aureus* containing protein A can absorb the blocking activity from serum of a tumor host. We devised an extracorporeal system consisting of a plasma cell separator and 0.2μ membrane filters in which intact *S. aureus* can be safely used as an immunoabsorbent.

The extracorporeal absorption of IgG and its complexes from serum of a tumor host resulted in reduction of serum-blocking activity and circulating immune complexes, appearance of cytotoxic antibodies, nonspecific stimulation of the immune system, and reduction and liquefaction of tumor mass.

INTRODUCTION

The presence of serum-blocking factors (SBF) in tumor hosts has been implicated as possibly inhibiting the effective antitumor cellular immunity *in vivo* (3,4,11). In several cases such blocking factors have been identified as immune complexes formed by circulating tumor antigens and antitumor antibodies (22). In some, perhaps in most, cases the antibody part of the circulating immune complexes (CIC) belongs to the IgG class (3,27,28).

The notion that SBF's are of importance in facilitating tumor growth has been supported by experiments in which passive infusion of xenogeneic "unblocking" serum, capable of counteracting the effects of blocking factors *in vitro*, resulted in tumor regression in various tumor models (5,8,9). However, xenogeneic unblocking serotherapy is limited in clinical situations by its potentially serious side effects (4).

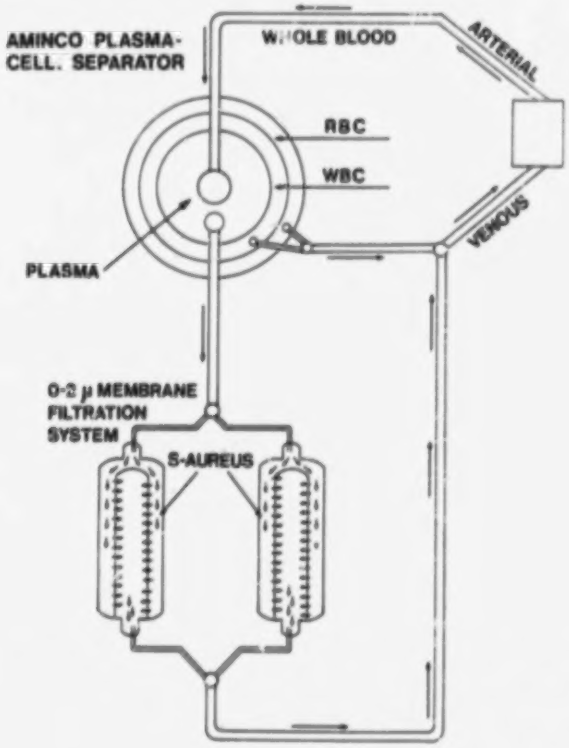
On the basis of the studies noted above, it has been repeatedly suggested that the role of SBF *in vivo* can best be evaluated from experiments designed specifically to remove these suppressive factors from the body fluids of tumor hosts for a protracted period of time (24). However, experience with the use of specific or nonspecific immunoabsorbents in an extracorporeal circulation is limited (6,26). The alterations in the cellular and noncellular components of blood and functions of vital organs of the host that are induced by the immunoabsorption procedures are virtually unknown. Further, their effect on the immune responsiveness of the host is also not known. Protein A, a molecule present on the wall of certain strains of *S. aureus*, has the biological property of interacting and binding to the Fc portion of mammalian IgG (23) and its complexes (16). Further, *S. aureus* containing protein A can absorb the blocking activity from serum of a tumor host (25). Over the past 5 years, we have developed an extracorporeal system in which formalin and heat-treated *S. aureus* can be safely used as an immunoabsorbent of IgG and its complexes (4,6). In addition, we studied some of the alterations induced in the immune system of the normal and tumorous hosts subjected to repeated *ex vivo* immunoabsorption procedures using *S. aureus* (6,7).

MATERIALS AND METHODS

An extracorporeal system consisting of a plasma cell separator and 0.2μ membrane filters was devised (6). A cell centrifuge (American Instrument Company) capable of separating anticoagulated blood into its cellular and plasma components was used. Those components were then pumped separately. In our procedure, the cells were returned unprocessed. The plasma was passed through an 0.2μ membrane filtration system containing *S. aureus*, as shown in figure 1. The details of extracorporeal circulation for dogs (6) and humans (7) have been described.

To prepare *S. aureus* Cowan I (NCTC 8530), *S. aureus* was grown in CCY medium, as described by Arvidson et al. (2), with slight modification. The organisms were killed and stabilized by heat and formalin treatment, as described by Kronvall (17). In each procedure, from 0.5 to 0.8 g wet weight of *S. aureus*/kg body weight of the host were used.

Figure 1.--Diagrammatic presentation of the extracorporeal immunoabsorption system


AMINCO PLASMA-CELL. SEPARATOR
WHOLE BLOOD
ARTERIAL
RBC
WBC
VENOUS
PLASMA
0·2 μ MEMBRANE FILTRATION SYSTEM
S-AUREUS

The safety of the extracorporeal system was established in 19 normal dogs subjected to 58 procedures, 49 using S. aureus and 9 without S. aureus.

Immunotherapy in Dogs With Spontaneous Tumors

Dog #1, which had a large ulcerated and fungating recurrent carcinoma (sweat gland) of the hind leg, was treated three times over a 2-week period. The tumor size was followed by xeroradiographic examination. Dog #2, bearing a large recurrent mammary tumor (adenocarcinoma) with extensive satellite metastases, was treated five times over a 3-week period. The tumor was followed by mammographic and histologic examinations. The possibility of ectopic hormone production in this dog was suggested by polydypsia (4 to 5 liters of water intake/day). Dog #30, bearing primary Leydig cell tumor of the testis with hypertrophy of multiple mammary tissues, was also treated five times over a 3-week period. Changes in tumor mass were recorded using standard mammographic techniques and by histologic examination.

Studies in Patients With Recurrent Colon Carcinoma

Two patients with recurrent adenocarcinoma of the colon were treated 19 and 8 times respectively. The detailed history and results of extracorporeal immunoabsorption procedures performed on the first patient have been published (7). The second patient, a 62-year-old man, developed a retroperitoneal mass about 2 years after palliative surgery for a perforated adenocarcinoma of the descending colon. At the second laparatomy, the mass was confirmed to be a recurrent adenocarcinoma by histopathologic examination. During an otherwise uneventful postoperative period, the patient developed a tumor-colo-cutaneous fistula. The patient was subjected to eight extracorporeal immunoabsorption procedures over a 6-week period. Alterations in tumor size were recorded by repeated ultrasound examinations. The patient received no other form of antitumor therapy before or after the original surgery.

RESULTS

Preclinical Safety Studies

Fifty-eight procedures were performed on 19 dogs without any morbidity or mortality. During each procedure we processed a volume equivalent to the expected plasma volume of each dog. From one to seven procedures were performed on each dog, over a period of 1 to 60 days. Nine of fifty-eight procedures were performed without *S. aureus* in the extracorporeal circulation, to serve as controls.

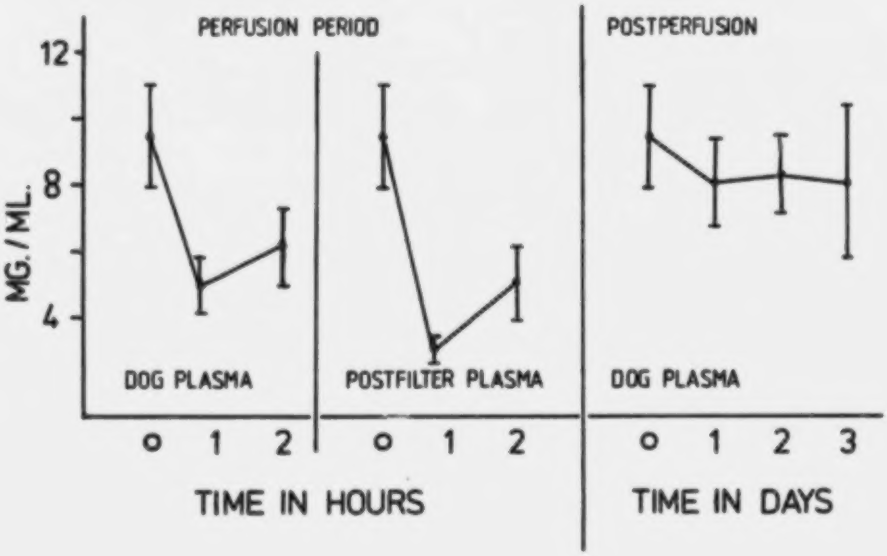
Thirteen procedures using *S. aureus* were performed to study the acute alterations in the blood pressure and electrocardiograms of the dogs. Slight increases in pulse rate, by 20 to 30 per minute; falls in blood pressure, by 10 to 15 mm of Hg; and occasional ectopic heart beats (on electrocardiogram) were observed during the procedure.

Thirty-six procedures using *S. aureus* were performed to study the biochemical, microbiological, and immunological changes in blood for the dogs. Further, the lymph nodes and kidneys of the dogs were histologically examined. The details of results have been published previously (6) and are summarized in table 1 and figure 2. A total of 50 specimens from 6 procedures, blood from dogs and plasma from extracorporeal circulation, were submitted for aerobic and anaerobic microbiological examination which showed no growth. Interestingly, a significant immunopotentiation, as evidenced by markedly proliferative lymph nodes and, often, rebound of serum IgG levels, was observed during the postperfusion period. Absence of surface antigens of staphylococcal origin was demonstrated in saline wash, collected prior to perfusion of plasma over the *S. aureus*, during nine procedures (6).

Table 1.--Safety studies in normal dogs

Parameters Studied During and After *Ex Vivo* Procedures in Each Dog	Results
Biochemical studies on blood from dog, and plasma from *ex vivo* circulation	Minimal and transient alterations
Pulse/blood pressure/EKG	Transient tachycardia during the procedure
Microbiological studies on blood from dog, and plasma from *ex vivo* circulation	No growth
Histological studies (kidneys and liver)	Normal

Figure 2.--Changes in IgG levels in normal dogs. The mean value with standard error for each time point is presented.



Immunotherapy Studies in Dogs with Tumors

In dog #1, after the second treatment, the tumor showed evidence of necrosis in the central part and epithelization near the margin in the ulcerated area of the tumor. The size of the tumor mass decreased appreciably (figures 3 and 4). Due to the poor general condition of the dog, no further treatments were given after the third procedure.

Figure 3.--Dog #1, xeroradiogram (pretherapy) showing soft tissue mass

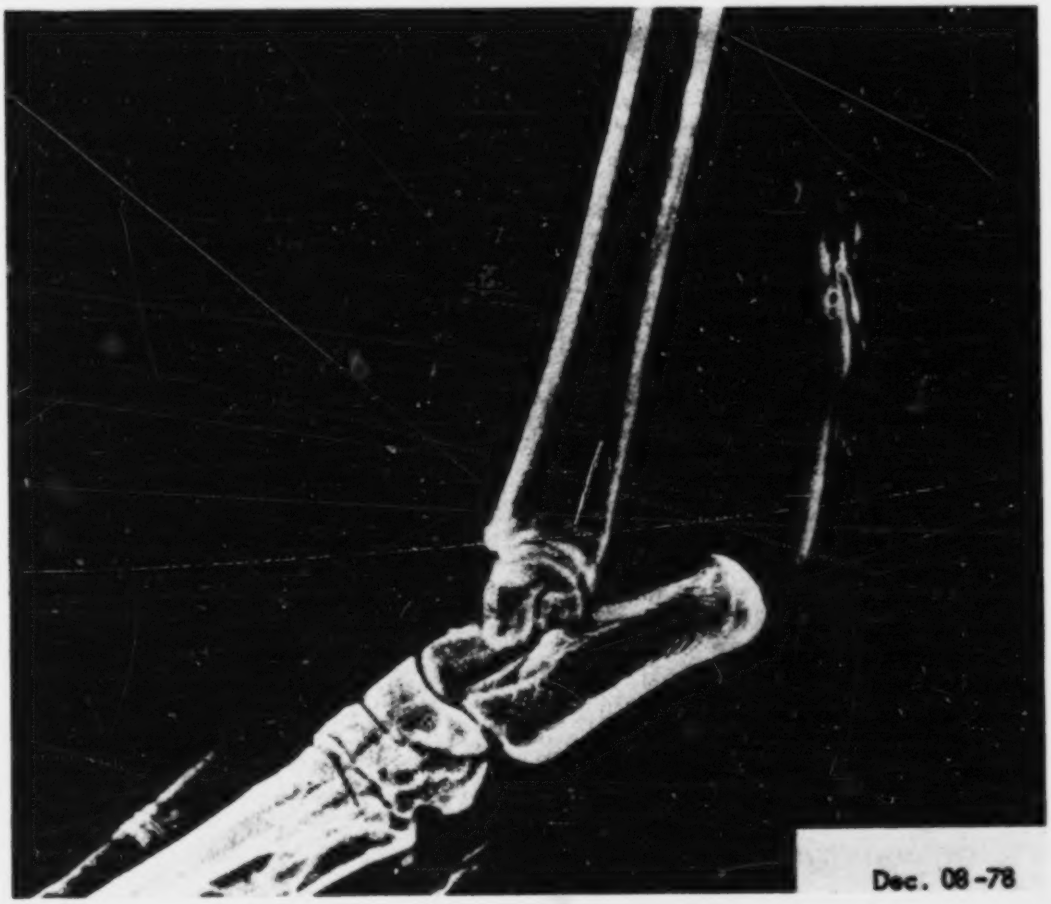
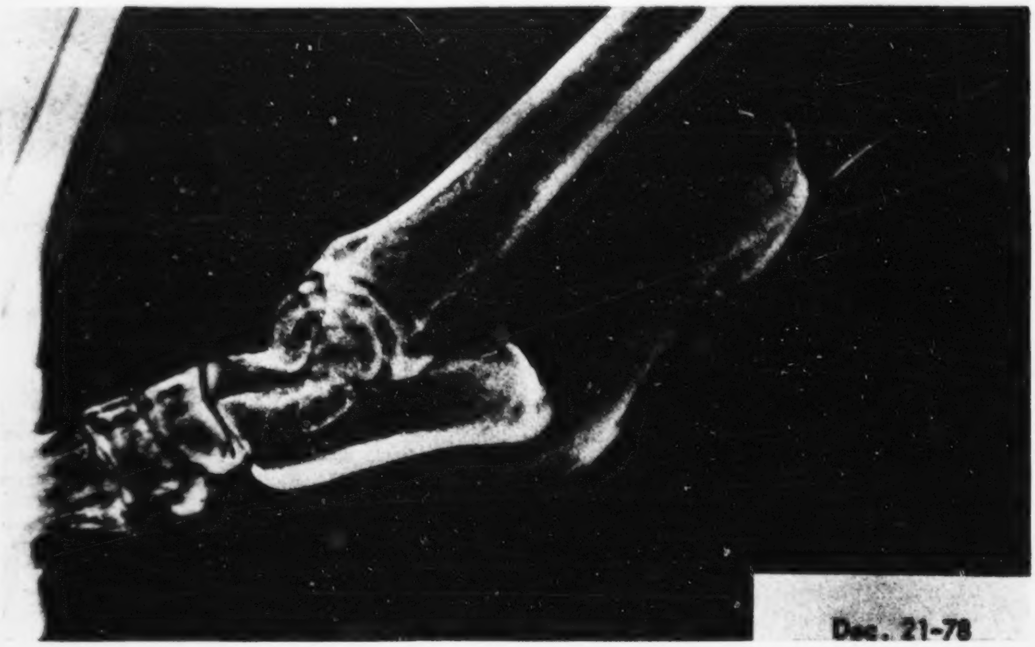


Figure 4.--Dog #1, xeroradiogram (after two treatments) showing reduction in the size of the soft tissue mass


Dec. 21-78

In dog #2, the significant alterations seen in the tumor were an increase in temperature on the surface of the tumor during each procedure and liquefaction of the tumor mass after two treatments. The mammographic examination showed an appreciable increase in calcification after the third treatment, and that increased further with two additional treatments (figures 5 and 6). The water intake of the dog abruptly returned to normal (1 liter/day) after the second treatment. The tumor mass, which was fixed to the underlying tissue, became mobile and soft, after the fifth treatment, at which time it was excised. The histologic examination confirmed the clinical and radiographic findings of necrosis and calcification (figures 7 and 8).

In dog #30, the tumor mass showed a significant decrease in size after four treatments (figures 9 and 10). The tumor mass was excised after the fifth treatment, and histologic examination showed evidence of necrosis and architectural changes (figures 11 and 12). The lymph nodes of dogs #2 and #30 showed proliferation of paracortical area and plasmacytosis, a finding consistently seen in normal dogs subjected to similar extracorporeal immunoabsorption procedures (6).

Figure 5.--Dog #2, mammogram (pretherapy) showing focal calcification in the tumor mass and satellite extension

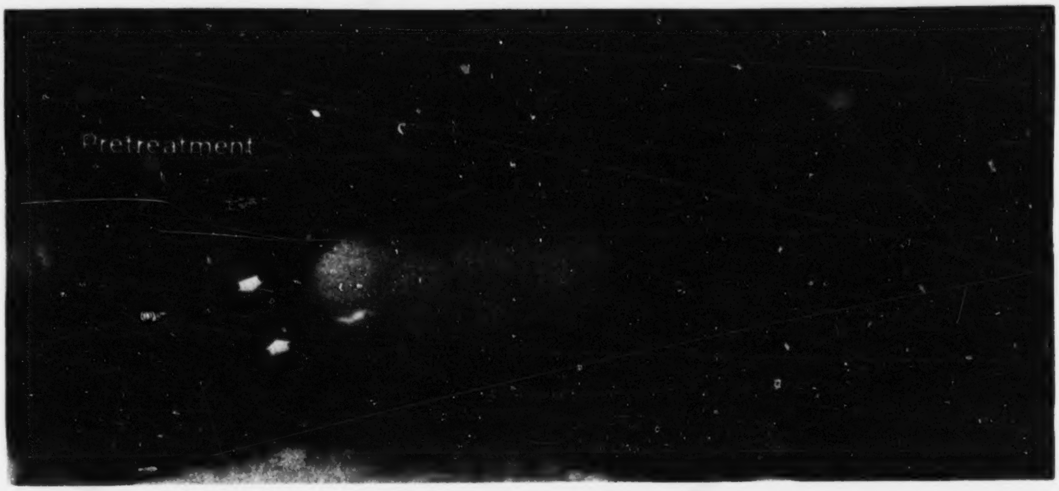


Figure 6.--Dog #2, mammogram (after four treatments) showing increase in calcification in the tumor mass and satellite extension. Notice how the area marked with arrows changed from a similar area so marked in figure 5.

Posttreatment

Figure 7.-- Large histological section through the whole mammary tumor mass. Dark parts in the periphery represent vital tumor cells, while the pale central parts represent necrotic areas. Htx - eos x 3.7

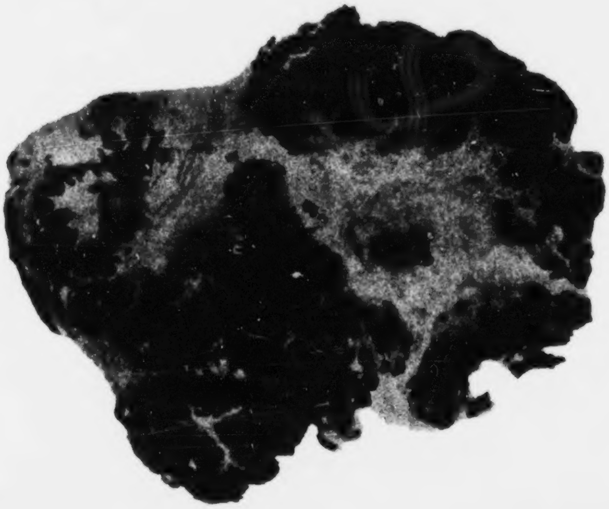


Figure 8.-- Dog #1, showing necrosis and calcification amid mitosis-rich tumor. Compare with mammogram, figure 6. Htx - eos x 320

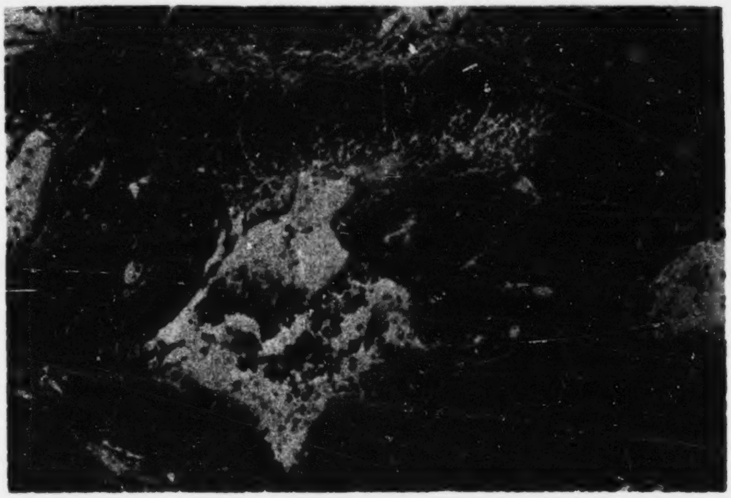


Figure 9.--Dog #30, radiogram using mammographic techniques (pretherapy), of the testis showing a solid tumor mass

Figure 10.-- Dog #30, radiogram using mammographic techniques (after three treatments) showing reduction in size of the tumor mass and changes in its contour

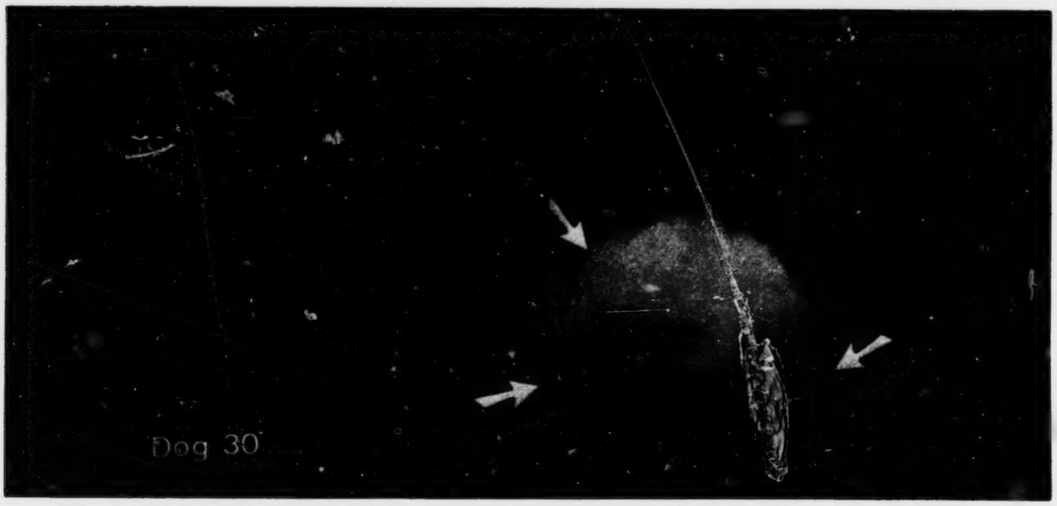


Figure 11.-- Dog #30, Leydig cell tumor of the testis, a rather cellular tumor composed of large Leydig cells rich in granular cytoplasm. Htx - eos x 320

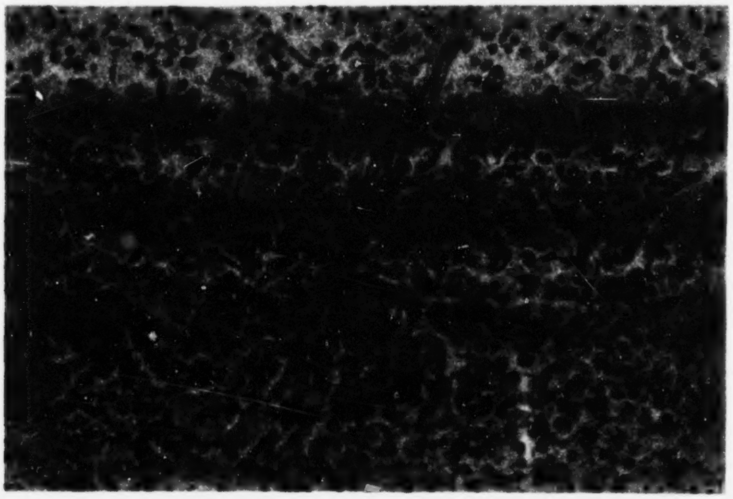
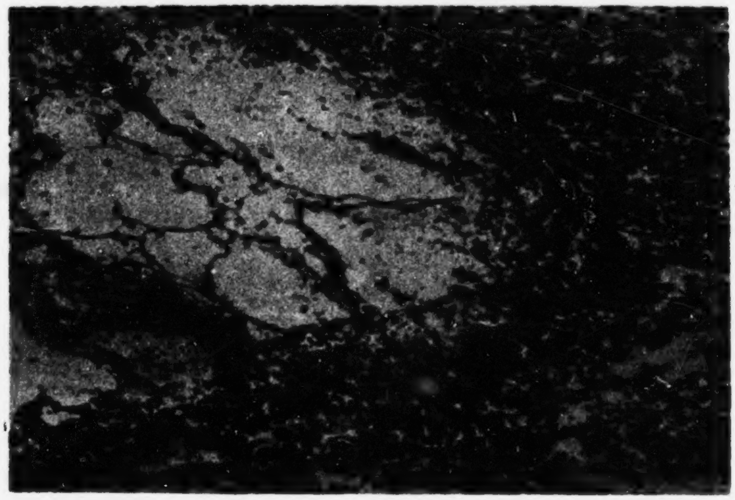


Figure 12.-- Dog #30, showing necrotic and degenerating areas in the tumor mass. Htx - eos x 125



Studies in Humans With Recurrent Colon Carcinoma

The details of the first patient have been published (7) and are briefly given in tables 2 through 4. The major clinical findings in the second patient were improvement in the general condition of the patient, decrease in size of the tumor (clinically), liquefaction of tumor mass (figure 13), and healing of tumor-colo-cutaneous fistula. The biochemical and immunological alterations were similar to those observed in the first patient. After eight treatments, no further extracorporeal immunoabsorption was performed on this patient, for reasons unrelated to the procedure.

Table 2.--Summary of clinical observations during extracorporeal perfusion

	Procedures Using *S. Aureus* (mean values from first five treatments)	Procedure Without *S. Aureus* (one treatment)
Duration of *ex vivo* circulation (in minutes)	106	108
Amount of *S. aureus*	52 g	0
Plasma volume processed (in liters)	1.28	1.0
Temperature on tumor surface--before treatment	35.3 °C	35.3 °C
—after treatment	39.9 °C	35.9 °C
Systolic blood pressure--high	128.0 mm	142.0 mm
—low	95.6 mm	95.6 mm
Intake--first 24 hours after treatment (in liters)	4.8	2.9
Output--first 24 hours after treatment (in liters)	4.0	2.1

Table 3.--Correlation of serum levels of IgG, Clq binding and blocking factors during and after extracorporeal absorption of IgG with S. aureus

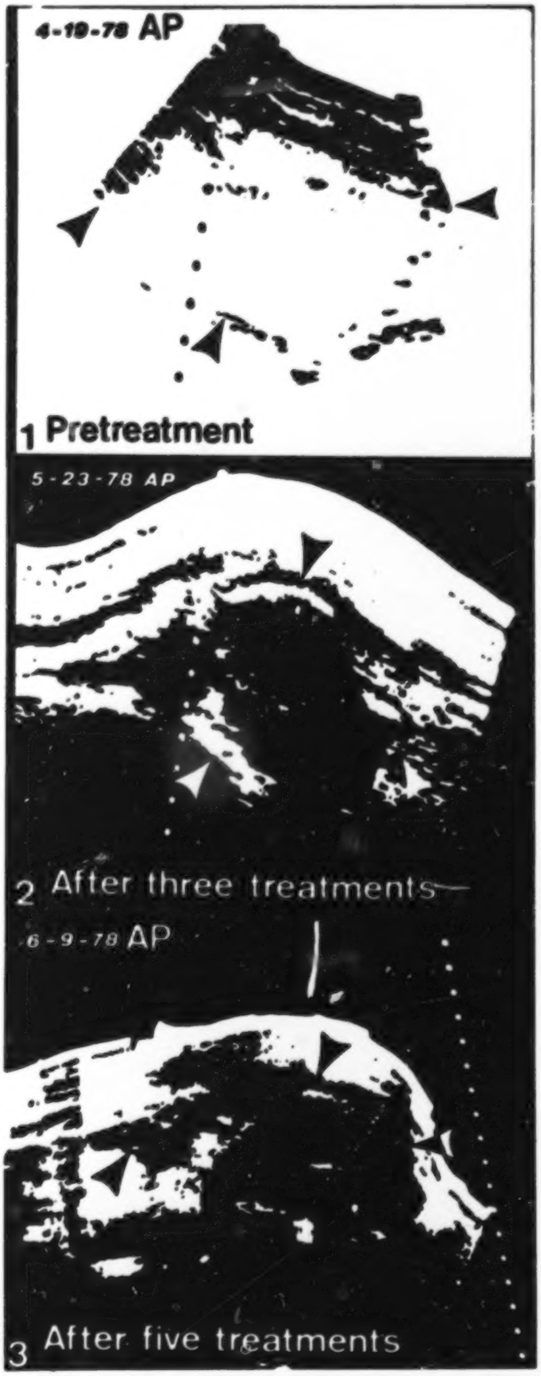
Specimen Description	IgG	Percent Clq Binding	Percent Blocking
Prerun	1,230	20.0	64.0
Endrun	825	10.2	12.3
24 hours postrun	975	30.3	6.3

[Serum blocking activity was tested by microcytotoxicity assay using autochthonous colon carcinoma target cells and peripheral blood lymphocytes (7).]

Table 4.--Summary of observations in a patient with colon carcinoma (metastatic) treated with extracorporeal immunoabsorption using S. aureus

1. Improvement in general condition of the patient
2. Healing of ulcerated areas in the tumor and decrease in tumor mass
3. Disappearance of peritoneal metastasis (on second laparotomy)
4. Increase in Ig-bearing lymphocytes and serum levels of IgM
5. Decrease in serum-blocking factors and appearance of complement-dependent cytotoxic antibodies
6. Histologic evidence of tumor destruction (necrosis, fibrosis, and calcification)

Figure 13.--Patient #2; sequential sonograms of the retroperitoneal mass, showing evidence of liquefaction and changes in its contour


4-19-78 AP
1 Pretreatment
5-23-78 AP
2 After three treatments
6-9-78 AP
3 After five treatments

DISCUSSION

Cellular and humoral antitumor immune responses can be demonstrated in tumor-bearing animals and humans. The inability of the tumor host to reject its tumor in the presence of sensitized lymphoid cells remains unexplained. It has been partly explained by the immunological defects identified in hosts with growing neoplasms. Two defects stand out in particular: a quantitative decline in immune competence with the establishment and growth of malignant tumors (12); and the appearance of factors capable of inhibiting antitumor cellular immunity (1,11,21). Therefore, procedures capable of either removing or counteracting the serum-blocking factors (antigens, antigen-antibody complexes, and antibodies), combined with methods bolstering specific and nonspecific immunity, could be expecced to result in optimal antitumor effects (4).

A few immunologic methods have been suggested (18,24) for reducing the load of free or complexed tumor antigens and levels of antitumor IgG_1 and IgG_2 (often claimed to enhance tumor growth) in a tumor host:

- SBF can be removed from the extracorporeal circulation of a tumor host by using specific immunoabsorbents.
- Circulating blocking factors can be reduced nonspecifically by plasmapheresis and replaced by plasma which does not have blocking activity (14). Similarly, lymphapheresis by thoracic duct cannulation can be used to remove lymph-containing blocking activity although in different tumor models it had both beneficial (13) and harmful effects (19) and essential plasma components were needed for proper nutrition of the host.
- It may be possible to dissociate the antigen-antibody complexes derived from serum of a tumor host and reinfuse the antibody fraction. This assumes that the dissociated antibody fraction is putatively beneficial.
- It may be possible to suppress the production of SBF by appropriate immunization.
- SBF might be counteracted by administration of unblocking serum raised either in allogeneic hosts or by hybridoma-producing specific antibodies. Alternatively, it may be possible to increase the levels of unblocking antibodies with appropriate immunization.
- It may be possible to suppress the production of SBF by appropriate immunization and use of immunosuppressive agents.

In vitro studies led to yet another method that might be able to nonspecifically reduce sufficient quantities of SBF repeatedly for a protracted period and that may also result in immunopotentiation (4,25). That possibility was based on the principle that certain strains of S. aureus containing protein A can combine with the Fc portion of mammalian IgG (23) or its complexes (16). It was speculated that serum-blocking factors could be purified protein A (4). However, to use either intact staphylococci or purified protein A as immunoabsorbents for SBF in an extracorporeal circulation, knowledge of effects of their usage on the functions of vital organs and plasma of the host was needed.

It can now be stated that the use of S. aureus as an immunoabsorbent is safe. Our studies strongly suggest that using S. aureus for absorption of IgG and its complexes from the circulation of tumor hosts can result in reduction of blocking activity, stimulation of immune response, and beneficial antitumor effects. It is of interest that use of the procedure in dogs with spontaneous tumors and in humans with recurrent tumors indicated numerous similarities, albeit our experience is limited.

It also practical to combine the procedure of ex vivo immunoabsorption of immune complexes with other methods, such as the use of nonspecific immunostimulants and adoptive transfer of in vitro treated lymphocytes of known biologic functions (10). As our understanding of the complex tumor-host relationship expands, multimodal immune manipulation is likely to become a more optimal approach than isolated procedures (8,10).

We are convinced that a more detailed investigation of various biological and well-defined immunoabsorbents for removal of immune complexes (20,27), antigens (11), and certain types of antibodies with specific and nonspecific effects on immune reactivity (11,21) is warranted. Further, studies in animal models, such as cats with spontaneous leukemia (15) and dogs with solid tumors, are needed to establish the mechanisms of immunotherapeutic effects. The experience gained by using such immunoabsorption procedures may not only be important because of their applications to tumor hosts, it also may have relevance to patients with autoimmune disease and to recipients of organ transplants.

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EXTRACORPOREAL IMMUNOADSORBENTS FOR THERAPY OF AUTOIMMUNE AND NEOPLASTIC DISEASE

David S. Terman, M.D.
Department of Internal Medicine, Baylor College of Medicine
Texas Medical Center
Houston, Texas

INTRODUCTION

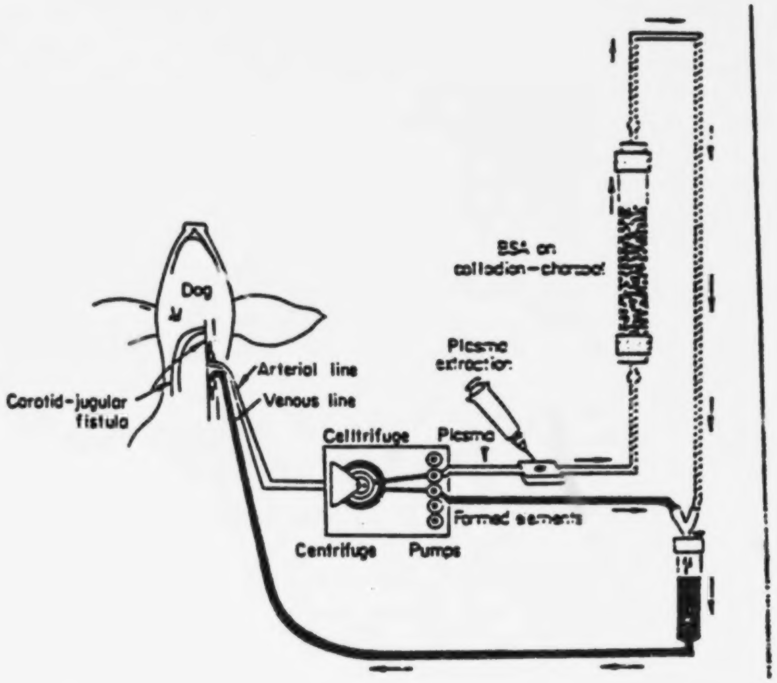
The role of immune reactants in many experimental and human diseases is now well established (10,36). Therapy for many of the immunologically mediated diseases has consisted largely of the use of pharmacologic agents that widely and nonspecifically suppress host immunity leading to numerous undesirable effects. With increasing awareness of the etiopathogenic factors in many immunologically mediated diseases, there has been the concomitant development of many sensitive radioimmunological techniques to measure those factors in serum. In this review, attention is focused on the development of various immunoadsorbents that have been designed to extract pathogenic immune reactants from the circulation. Several immunoadsorbents consisting of immobolized antigens, antibodies, and enzymes have been developed. When placed in an extracorporeal circuit, those immunoadsorbents have shown a capacity to extract or hydrolyze immune reactants in the circulation without demonstrable release of immobilized substances or significant immediate or long-range toxicity to the host. Recently, various extracorporeal immunoadsorbents have been employed to treat several experimental and human disease processes; initial findings are promising.

PROPERTIES OF IDEAL IMMUNOADSORBENT FOR IN VIVO USE

An immunoadsorbent designed to extract immune reactants from circulating plasma *in vivo* must have several important characteristics. First, it would be ideal to employ a biocompatible solid support that may be devised for covalent coupling or physical entrapment of immune reactants. Second, the support should present a large surface to volume ratio so that a small extracorporeal volume of plasma may contact a large immunoadsorbent surface. Third, the material should be capable of binding large quantities of immune reactants, and the binding should be sufficiently firm to prevent release of the immobilized substances into the circulation. Fourth, both the immune reactant and the base support should be structurally stable, to avoid embolization of structural material into the host. Fifth, the support and immobilized immune reactants should be minimally thrombogenic, to prevent clotting. Finally, the support system should have low nonspecific adsorption characteristics and allow adequate flow rates.

It may also be desirable to selectively perfuse immunoadsorbents with cell-free plasma rather than whole blood to avoid the release of any vasoactive substances from platelets and leukocytes that might be activated after fixation of complement to immune reactants on the surface of the immunoadsorbent. Hence, a necessary step prior to safe and effective immunoadsorption might be to separate whole blood into formed elements and plasma in a cell-separation unit and then allow only plasma to selectively perfuse the immunoadsorbent system. Figure 1 is a schematic representation of an extracorporeal immunoadsorbent system we have employed for several of our studies.

Figure 1.--Extracorporeal immunoadsorbent system employed for numerous studies in the author's laboratory


BSA on
collodion-charcoal
Dog
Plasma
extraction
Arterial line
Venous line
Carotid-jugular
fistula
Celltrifuge
Plasma
Formed elements
Centrifuge
Pumps

SPECIFIC REMOVAL OF CIRCULATING ANTIBODIES WITH IMMOBILIZED ANTIGENS

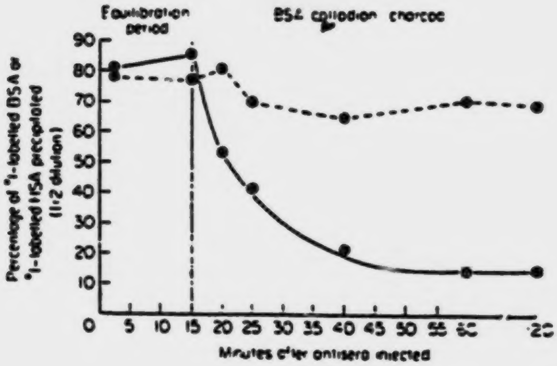
Graf et al. (12) were the first to show that immunoadsorbents could be employed to selectively remove antibodies from actively and passively immunized rabbits. In later studies, Schenkein et al. (19) developed an extracorporeal immunoadsorbent system in which bovine serum albumin (BSA) was immobilized in agarose and proved capable of selectively removing BSA antibodies from the circulation. A similar immunoadsorbent in which ssDNA antigen was immobilized proved capable of extracting ssDNA antibody in both passively and actively immunized rabbits (31). Because of the fragility of the supporting matrix and the possibility of leaching of immobilized substances, new and more stable extracorporeal immunoadsorbents were subsequently developed.

Various antigens, such as BSA, DNA, and glomerular basement membrane (GBM) extract, have been immobilized on several solid supports and have been employed as extracorporeal immunoadsorbents (25,26,27,32, 26,34). Those antigens have been physically adsorbed or entrapped in cellulose nitrate membranes adherent to small charcoal particles by a modification of procedures described by Chang (9). Collodion, ether, and alcohol are placed in a beaker containing antigen dissolved in tris buffer. To that solution, activated coconut charcoal is added. The charcoal is gently stirred under a vacuum hood until the particles are dry. The antigen-coated charcoal particles are then spread out evenly on a glass plate to permit the ether to evaporate. They are then packed into a prefilled chromatographic column and washed extensively with normal saline solution.

The protein antigen BSA, as well as DNA and GBM, was immobilized in collodion charcoal in this fashion (25,26,27). Using radioactive antigens as markers, we have been able to quantitate the uptake of those substances in collodion charcoal and to determine their stability on the charcoal during extracorporeal circulation studies *in vivo* by direct measurement of radioactivity on the columns before and after perfusion experiments. All three antigens have been firmly embedded in collodion charcoal with no demonstrable release of bound materials into the host.

To determine whether the immobilized antigens would retain their capacity to combine with their respective antibodies *in vivo*, they were placed in an extracorporeal circulation system on line with a plasma-cell separator. In representative studies, antisera to human serum albumin (HSA) and to BSA were passively infused into adult mongrel dogs, and, after an equilibration period of 15 minutes, canine plasma was pumped over BSA collodion charcoal at a flow rate of 20 ml/minute. That resulted in an abrupt and specific decline in BSA antibody with minimal change in HSA antibody over the same time period (31) (figure 2).

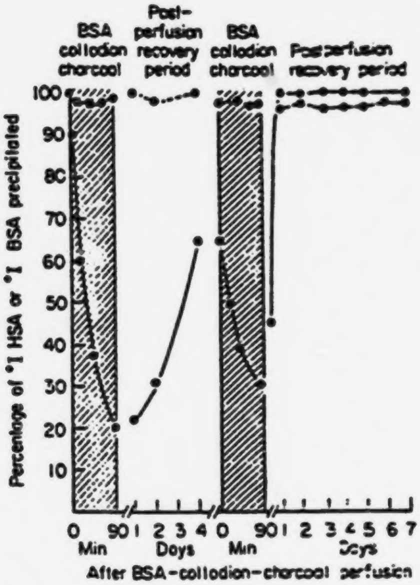
Figure 2.--Dogs were infused with anti-BSA (——) and anti-HSA (---) antisera. After an equilibration period of 15 minutes, plasma was circulated over BSA collodion charcoal at a flow rate of 40 ml/min. There was significant reduction in BSA antibody over the same time period.



Mongrel dogs were then actively immunized to BSA and HSA. After perfusion over BSA collodion charcoal there was an 80 percent decline in BSA antibody with a rebound in the postperfusion period that reached preperfusion levels in 7 days. Retreatment resulted once again in a specific decline in BSA antibody; however, the kinetics of antibody rebound in the postperfusion period was observed to be variable, and, in some instances, the rebound level exceeded the preperfusion level within 24 hours after the immunoadsorbent treatment (27,24) (figure 3). The amount of HSA antibody was unchanged over the same time period. As in other studies, there was no evidence of release of ^{125}I BSA from the immunoadsorbent, and there was no acute or chronic toxicity to the host.

The capacity to immobilize calf thymus DNA was studied. DNA antibodies, when combined with specific antigen, form immune complexes that are deposited in tissues and create inflammation. Indeed, DNA: anti-DNA immune complexes are considered to be the major pathogenic immune complex in systemic lupus erythematosus (SLE). Initially, DNA was physically immobilized in agarose after first being absorbed in powdered cellulose. That immunoadsorbent proved capable of specifically extracting ssDNA antibodies from the circulation of actively and passively immunized rabbits (22).

Figure 3.--Two sequential perfusions over BSA collodion charcoal were carried out in the same dog. After the second treatment, rebound of BSA binding was more abrupt than observed after the first perfusion (---) HSA; (—) BSA.

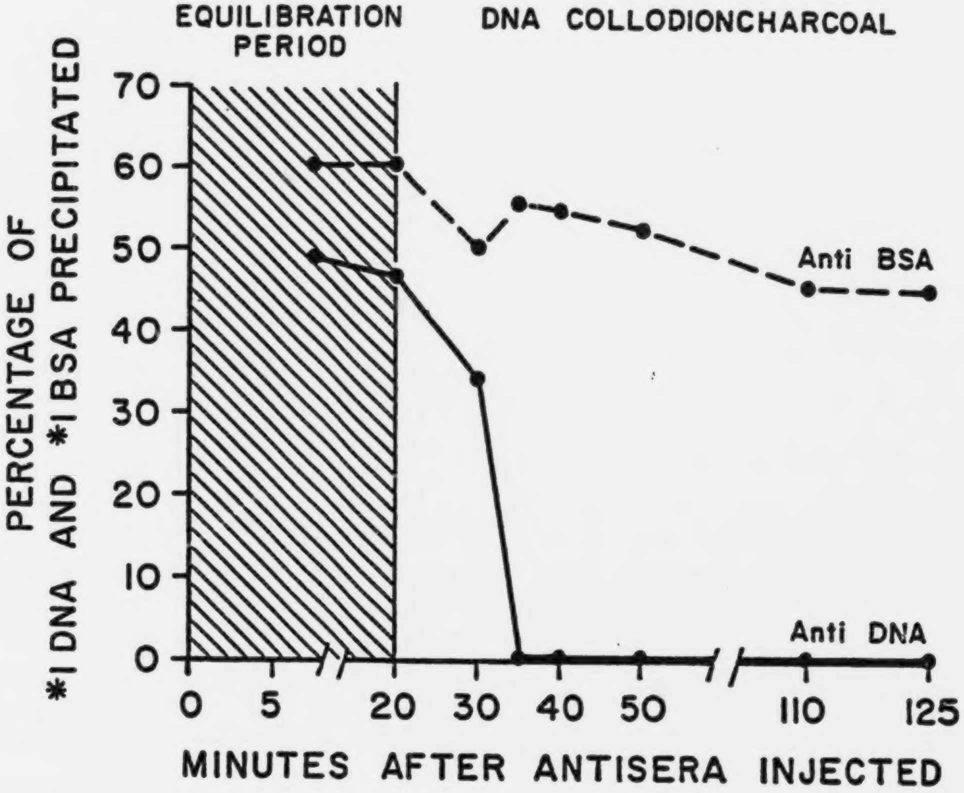


An immunoadsorbent more applicable for human use was developed when DNA was immobilized in collodion charcoal (26). Up to 7 mg of calf thymus DNA were immobilized in collodion charcoal which was then capable of specifically removing anti-DNA antibodies that had been passively infused into mongrel dogs. After an equilibration period of 20 minutes, there was an abrupt and specific decline in DNA antibody in plasma after passage over DNA collodion charcoal; there was minimal change in BSA antibody over the same time period (26) (figure 4). The method was employed to treat a single patient with severe SLE, and a similar decline in DNA binding was observed as will be described later.

SUPPRESSION OF ANTIBODY REBOUND AFTER EXTRACORPOREAL IMMUNOADSORPTION

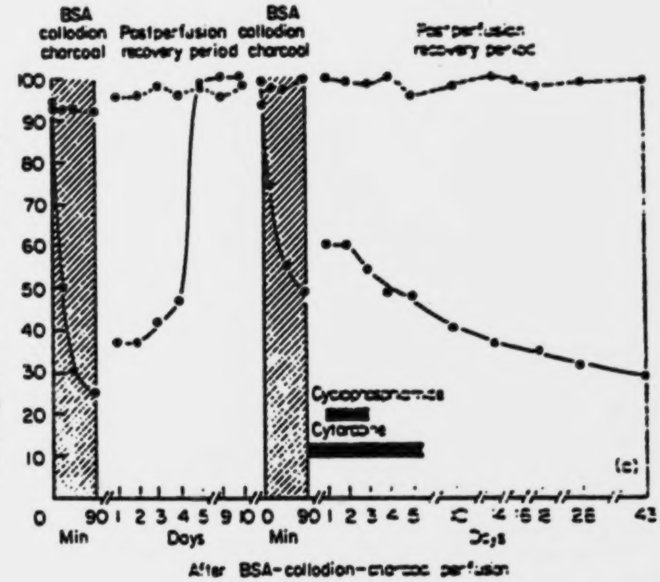
An exploration of measures to modify the antibody rebound following specific antibody removal with extracorporeal immunoadsorption employed various chemotherapeutic agents. Mongrel dogs were actively immunized to BSA and HSA, and 7 to 12 weeks later each had comparable titers of anti-HSA and anti-BSA antibodies. Control extracorporeal immunoadsorption was carried out in each dog, and its own pattern of postperfusion rebound was recorded. After a second extracorporeal perfusion, single-drug chemotherapy with cyclophosphamide, methotrexate, or cytosine

Figure 4.--Results of a typical extracorporeal perfusion study. Anti-DNA and anti-BSA were injected intravenously, and, after an equilibration period of 20 minutes, plasma was pumped over DNA collodion charcoal immunoadsorbent. There was an abrupt decline in DNA binding over the ensuing 15 minutes with minimal change in BSA binding over the same time period. DNA and BSA antibodies were both measured at 1:2 dilution of canine antiserum.



arabinoside resulted in a slight delay or had no effect on the recovery of antibody to preperfusion levels. In contrast, dual chemotherapy with cyclophosphamide and cystosine arabinoside resulted in specific attenuation or arrest of BSA antibody rebound (34). The immunosuppressive effect was specific since control levels of HSA antibody were only minimally changed by the extracorporeal immunoadsorption and the chemotherapeutic treatment (figure 5). Drug toxicity was transient and reversible upon cessation of treatment, but the immunosuppressive effect lasted well beyond discontinuation of the drug treatment (34).

Figure 5.--After extracorporeal immunoadsorption over BSA collodion charcoal, BSA antibody was specifically reduced. The postperfusion use of cytarabine and cyclophosphamide resulted in specific suppression of BSA antibody levels compared to the control rebound period. HSA antibody was unaffected by the extracorporeal adsorption and the cycle-dependent immunosuppressive agents. (—) BSA, (---) HSA antibody.



The sequence of events leading to the specific immunosuppression observed with cyclophosphamide and cytosine arabinoside have been as follows: a) BSA antibody-producing cells were recruited to proliferate after specific BSA antibody extraction with extracorporeal immunoadsorption; b) cytosine arabinoside given in the immediate postperfusion period may have partially synchronized the BSA antibody-producing cells in the S phase of their proliferative cycle; c) cytosine arabinoside and cyclophosphamide employed together on days 1, 2, and 3 were effective in killing the synchronized BSA antibody-producing cells, leaving nonproliferating HSA antibody-producing cells intact, thus producing a specific immunosuppressive effect. Such a principle might be of potential value in numerous acute and chronic disease states in which antibodies play an important pathogenic role. With sensitive techniques now available for monitoring circulating antibodies, the appropriate point at which to introduce extracorporeal immunoadsorption to remove pathogenic antibodies might be determined, and, with sensitive markers of proliferative activity of antibody-producing cells, the precise timing for chemotherapy might also be ascertained. Those measures combined could provide an effective basis for specific immunotherapy of numerous antibody-mediated diseases in man.

SPECIFIC REMOVAL OF CIRCULATING ANTIGENS WITH IMMOBILIZED ANTIBODIES

It is well recognized that circulating antigens play an important role in the pathogenic sequence in many experimental and human diseases. Soluble circulating antigens may combine with antibody to form soluble antigen-antibody complexes which may be deposited in tissues, resulting in inflammation. In addition, circulating soluble antigens may have a role in blocking cell-mediated immune function, thus initiating or perpetuating some neoplastic processes. To selectively remove antigen from the circulation, antibody was immobilized in collodion charcoal in a fashion similar to that described for antigens above. For *in vivo* testing, an extracorporeal circulation system was established in which arterial blood was passed through a continuous-flow cell separator, and plasma was circulated over anti-BSA collodion charcoal and control rabbit gamma globulin collodion charcoal (figure 6). ^{125}I-BSA was passively infused into mongrel dogs, and plasma was then circulated over the extracorporeal immunoadsorbents for 2 hours. Results showed up to 896 percent greater uptake of circulating ^{125}I-BSA on the charcoal containing immobilized anti-BSA compared to the control charcoal (28) (table 1). There was no evidence of release of anti-BSA from the immunoadsorbent since ^{131}I-anti-BSA cpm on the charcoal before and after experiments was unchanged.

Additional studies showed that purified BSA antibodies could be covalently linked to albumin-coated cuprophan membranes that were activated with glutaraldehyde. BSA antibody-coated cuprophan columns withdrew nearly four times as much circulating ^{125}I-BSA from plasma as did a corresponding control column to which rabbit IgG was covalently coupled (29).

HYDROLYSIS OF CIRCULATING SUBSTRATE WITH IMMOBILIZED ENZYMES

In some instances, it might be desirable to degrade pathogenic substances in the circulation by using enzymes immobilized on an inert solid support. In systemic lupus erythematosus, the degradation of circulating nDNA or of nDNA:anti-DNA immune complexes might be a desirable therapeutic objective. To accomplish it, nylon microspheres approximately 1.0 mm in diameter were prepared. They were activated by acid hydrolysis and glutaraldehyde treatment after which the enzyme deoxyribonuclease I (DNAase I) was covalently conjugated to them (30). DNAase I is a heat-stable exonuclease which is capable of cleaving DNA into lower molecular weight fragments by hydrolyzing phosphodiester linkages. For *in vivo* studies, DNAase I microcapsules were placed in an extracorporeal chamber (figure 7). Mongrel dogs were

Figure 6.--Schematic representation of an extracorporeal immunoadsorption system for removal of circulating antigen, employing antibody immobilized in collodion charcoal

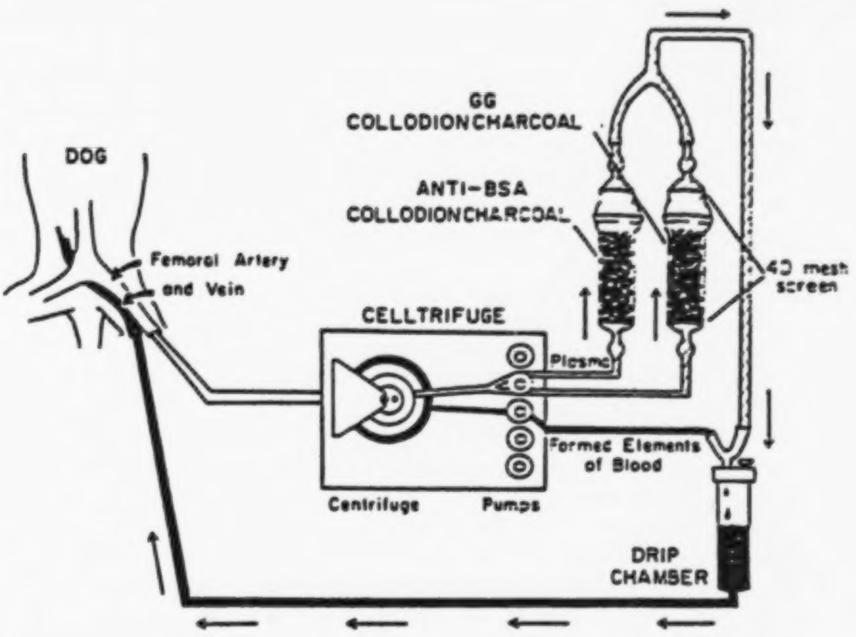
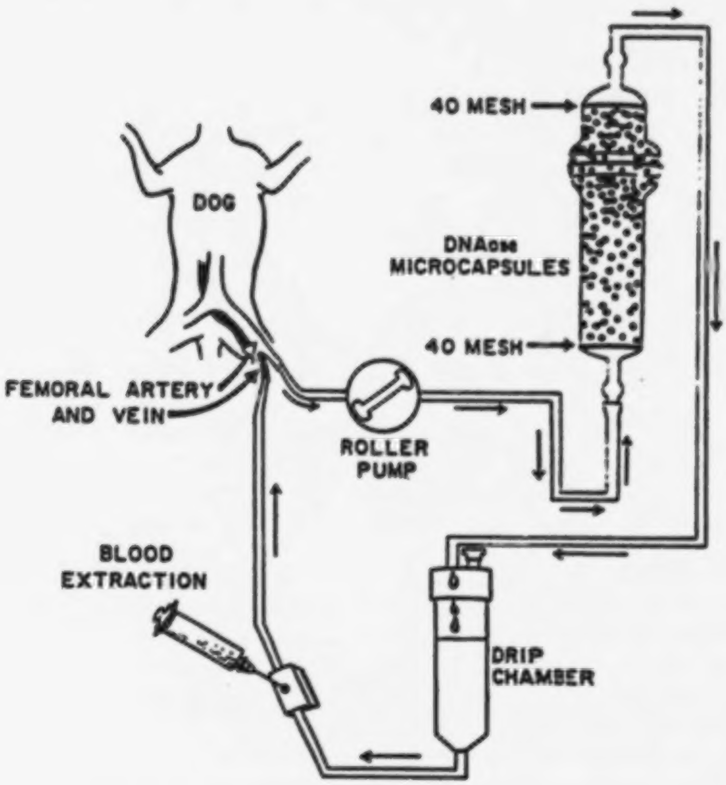

GG
COLLODIONCHARCOAL
DOG
ANTI-BSA
COLLODIONCHARCOAL
Femoral Artery
and Vein
40 mesh
screen
CELLTRIFUGE
Plasma
Formed Elements
of Blood
Centrifuge
Pumps
DRIP
CHAMBER

Table 1.--Uptake of ^{125}I-BSA on collodion charcoal[a]

Dog Number	Anti-BSA Collodion Charcoal		Normal Gamma Globulin Collodion Charcoal	
	cpm	μg	cpm	μg
in vitro	52,334	1.30	6,441	0.16
1	388,688	9.71	43,355	1.08
2	271,689	6.79	37,688	0.94
3	251,826	6.29	88,838	2.22

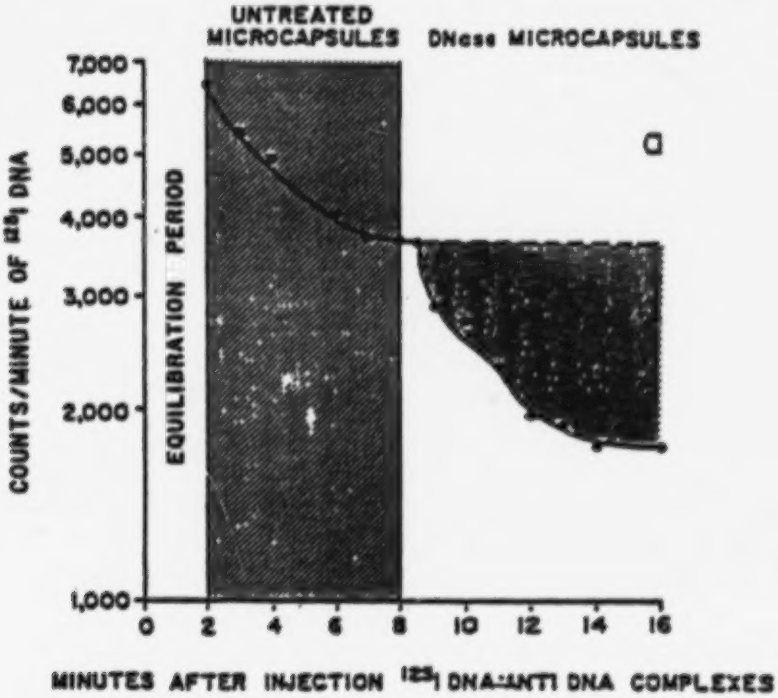
[a] Approximately 1.5 x 10^6 cpm of BSA were found to be circulating in each animal based on cpm in 1-ml plasma sample taken 5 minutes after injection of ^{125}I-BSA extrapolated for total plasma volume of each dog calculated at 4.98% of total body weight. At the conclusion of *in vitro* or *in vivo* circulation studies, collodion charcoal was washed with 400 ml of 0.15 M NaCl and counted in a gamma scintillation counter.

Figure 7.--Schematic representation of the *in vivo* extracorporeal system in which DNAase was covalently coupled to nylon microspheres



injected with ^{125}I-nDNA, and a variation in initial half-life in individual animals was observed. Therefore, for experimental studies, ^{125}I-nDNA was injected, and the decay in plasma was recorded during a control period in which untreated microcapsules were utilized in the extracorporeal system. DNAase I microspheres were then introduced into the extracorporeal circuit, which resulted in an acceleration of degradation of acid-precipitable ^{125}I-nDNA. ^{125}I-nDNA:anti-DNA complexes were passively infused into dogs, and, after a similar control period of circulation over untreated microcapsules, DNAase I microspheres were introduced. The results showed a rapid acceleration in the degradation rate of ^{125}I-nDNA:anti-DNA complexes precipitable with $(NH_4)_2SO_4$ (33) (figure 8). There was no substantial hematologic or biochemical toxicity to the host. Hence, the system may have a potential role in the specific therapy of SLE, when it is desirable to hydrolyze DNA circulating free or in combination with antibody.

Figure 8.--In this representative study, dogs were infused with ^{125}I-DNA: anti-DNA complexes. There was an increase in the rate of nDNA degradation in the immune complexes after placement of DNAase I microcapsules in the extracorporeal circulation. The points from 9 to 16 minutes are at least 12 SD below their predicted values. Interrupted lines represent extrapolations of each animal's control decay curve into the experimental period.



REMOVAL OF CIRCULATING IMMUNE COMPLEXES

Circulating immune complexes have now been implicated in the pathogenesis of numerous autoimmune diseases (22). Their presence in the circulation often correlates with disease activity, and they may be found deposited in tissues (39). In addition to the studies described above for hydrolysis of nDNA:anti-DNA complexes, preliminary work has shown that Clq, the first component of complement, will bind to immune complexes and may be immobilized in collodion membranes. The membranes were prepared by the method used by Carr and Sollner (6) and were composed of (a) a base layer of collodion and (b) a thin layer of collodion containing Clq (28). When placed in an extracorporeal circulation system in parallel with control membranes, the Clq collodion membranes were capable of extracting circulating BSA:anti-BSA immune complexes that had been passively infused into dogs (28). Recently, bovine conglutinin has been isolated and immobilized via N-hydroxysuccinimide-mediated bonds to two solid supports. Conglutinin is avid for glycoprotein residues of C3 in immune complexes, and, once immobilized, is capable of rapidly and efficiently extracting preformed BSA:anti-BSA complexes from serum circulated over it at physiologic temperature and pH conditions. Conglutinin is readily and economically available, quite stable biochemically, and hence may be a promising instrument for the extraction of circulating immune complexes *in vivo* in various disease states (18).

The preliminary studies described are promising, and further work is required. Indeed, it has recently been suggested that the reticuloendothelial system may be blocked in certain autoimmune diseases and that when immune complexes are removed by plasmapheresis, the reticuloendothelial system may then recover, permitting more effective clearance of remaining complexes (11,15,16). Hence, the selective removal of immune complexes from the circulation by extracorporeal adsorption may be a desirable and attainable therapeutic goal.

ATTENUATION OF EXPERIMENTAL DISEASES BY EXTRACORPOREAL IMMUNOADSORPTION

Nephrotoxic Glomerulonephritis

Canine glomerular basement membrane was isolated, purified, and immobilized in collodion charcoal by methods similar to that used for DNA and BSA (25). For *in vivo* studies, an extracorporeal circulation system was established, and mongrel dogs were injected with rabbit-canine GBM antibodies. After perfusion over GBM collodion charcoal, renal tissue was examined by fluorescence and light microscopy for deposition of heterologous gamma globulin and glomerular inflammation both before and after the extracorporeal procedure. In control experiments, perfusion was carried forth over collodion charcoal with an unrelated immobilized antigen, namely HSA. Results showed less deposition of heterologous IgG and less inflammation in the kidneys of the experimental dogs than in kidneys of the controls. There was no evidence of release of ^{131}I-GBM from the immunoadsorbent, and there were no significant biochemical or hematologic alterations (25). Hence, the extracorporeal immunoadsorbent treatment was capable of attenuating the early heterologous phase of passively induced canine nephrotoxic nephritis.

This mode of therapy may well be applicable to patients with Goodpasture's syndrome, a disease that is mediated by anti-GBM antibodies in the circulation. Indeed, it appears that, after removal with plasmapheresis, GBM antibody levels in serum may not show substantial rebound in some cases (15). Hence, the efficient removal of GBM antibody from circulation with an extracorporeal immunoadsorbent might lead to rapid amelioration of the disease process. The recent development of a new convenient radioimmunoassay for measuring GBM antibodies in human sera should facilitate the quantitative assessment of immunoadsorbent capacity and permit serologic monitoring of the effectiveness of therapy (4).

Hyperacute Renal Xenograft Rejection

The major barrier to transplantation of organs between different species is the presence of naturally occurring, preformed donor-specific antibodies that are capable of rejecting transplanted organs in a rapid and efficient manner. An approach to attenuating that process might be to remove the preformed antibodies by immunoadsorption.

In preliminary studies, we determined that the complete expression of hyperacute renal xenograft rejection depends upon the presence of both humoral antibodies and formed elements of blood. Basing our theory on those findings, we predicted that hyperacute rejection would be markedly attenuated if porcine kidneys were perfused *in vivo* with canine plasma that was selectively depleted of formed elements. Prolonged function of porcine kidneys was observed during continuous extracorporeal perfusion with oxygenated canine plasma that was depleted of cellular elements by a plasma cell separator (22,37,38). Figure 9 is a schematic representation of that extracorporeal system. Indeed, porcine kidneys that were sequentially perfused with canine plasma showed progressive reduction in glomerular deposition of canine IgG, IgM, C3, and fibrinogen. A sensitive radioimmunoassay employed to detect porcine-specific canine antibodies showed up to 90 percent reduction of those antibodies after sequential plasma perfusion (22,37) (figure 10). At this point, all blood was returned to the dogs, and fresh porcine kidneys were then perfused with whole canine blood either *ex vivo* or by *in situ* transplantation (25). After whole-blood perfusion, hyperacute rejection was not observed, and the kidneys produced substantial quantities of urine for up to 8 hours after transplantation. Sequential immunofluorescent studies of kidneys perfused with whole blood showed progressive increase in glomerular deposition of canine IgG, IgM, C3, and fibrinogen (22). The dogs were not given immunosuppressive agents to suppress the rebound of heterospecific antibodies. However, the use of such agents, coupled with extracorporeal immunoadsorption, may result in even greater prolongation of renal xenograft survival.

Spontaneous Canine Breast Adenocarcinoma

After the initial observations made by Bansal et al. of tumoricidal effects after extracorporeal perfusion over *Staphylococcus aureus* Cowans I (27), an intensive trial of that therapy was begun in dogs with spontaneous mammary adenocarcinoma, a disease that in dogs is considered to be an excellent model of human breast cancer (23).

Extracorporeal perfusion of plasma from dogs with spontaneous canine breast adenocarcinoma over *Staphylococcus aureus* Cowans I (SAC)

Figure 9.--Schematic representation of extracorporeal circulation system to permit selective plasma perfusion of porcine kidneys

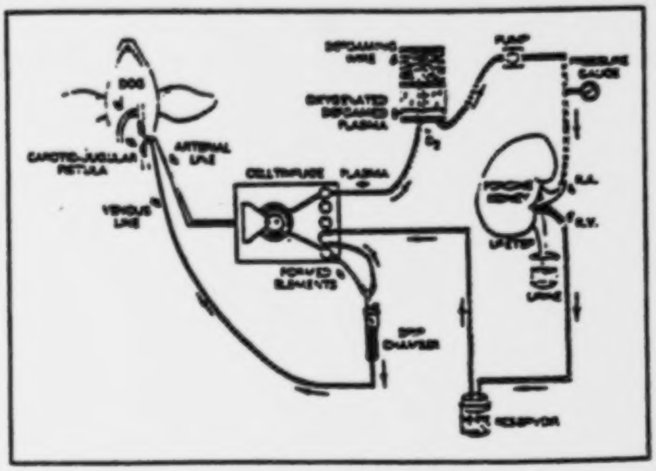
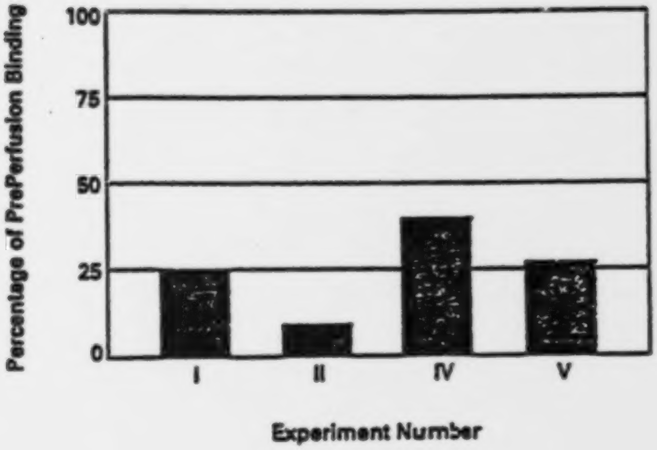

DOG
CAROTID-JUGULAR FISTULA
ARTERIAL LINE
VENOUS LINE
CELLTRIFUGE
PLASMA
FORMED ELEMENTS
DRIP CHAMBER
DEFOAMING WIRE
OXYGENATED DEFOAMED PLASMA
O_2
PUMP
PRESSURE GAUGE
PORCINE KIDNEY
R.A.
R.V.
URETER
RESERVOIR

Figure 10.--Decline in circulating porcine-specific canine antibodies after sequential extracorporeal perfusion of porcine kidneys with canine plasma prior to ex vivo whole-blood perfusion or transplantation.



has resulted in extensive necrosis of tumor at both primary and metastatic sites. In 14 dogs studied, changes were observed within 12 hours after perfusion. Repeat perfusion in 6 cases resulted in greater tumoricidal effect and healing of large ulcerating lesions. By 4 to 24 hours after perfusion, tumor cells showed diffuse necrosis associated with immunohistochemical deposition of canine IgG and C3 on tumor cell membranes. Ultrastructurally, the earliest and most consistent lesion in the tumor cells was tumor cell membrane lysis with intact nuclear membranes (23). At 24 to 48 hours after perfusion, there was extensive infiltration of necrotic areas by leukocytic elements. Serologically, tumor-specific antibodies assayed by indirect immunofluorescence were detected in serum by 4 hours after perfusion. IgG levels were reduced 20 to 30 percent after treatment, which was followed by a rebound that generally brought the level above the preperfusion level over the ensuing 24 hours. Circulating immune complexes (measured by solid phase Clq binding) declined during perfusion but rebounded so that the level exceeded preperfusion and remained above preperfusion levels for 72 hours. C3 levels declined below preperfusion values after treatment and were sustained for up to 48 hours. When ^{125}I SAC with unlabeled SAC was used in the extracorporeal filtration system there was no evidence of release of immobilized SAC from the membrane filtration system during extracorporeal perfusion. Clinical toxicity, which included mild fever and leukocytosis, was transient and abated without pharmacologic treatment. Four dogs treated by perfusion over Staphylococcus aureus Woods 46 (SAW) (nonprotein A bearing) showed no tumoricidal response. Each of those dogs showed a profound tumoricidal response to subsequent perfusion over SAC. In contrast to SAC treatment, with SAW there were no changes in tumor-specific antibody levels or rebound of IgG; there was no sustained reduction in C3 or elevation of Clq binding (23).

Morphologic and serologic findings after extracorporeal perfusion over SAC suggest that the acute tumoricidal response observed may be initiated by complement-dependent, tumor-specific antibodies which are either rendered operational or freshly generated by extracorporeal perfusion over SAC. The tumoricidal effect is rapid, extensive, and specific for primary and metastatic sites, with minimal host toxicity. This may represent a promising new immunotherapeutic approach to breast adenocarcinoma.

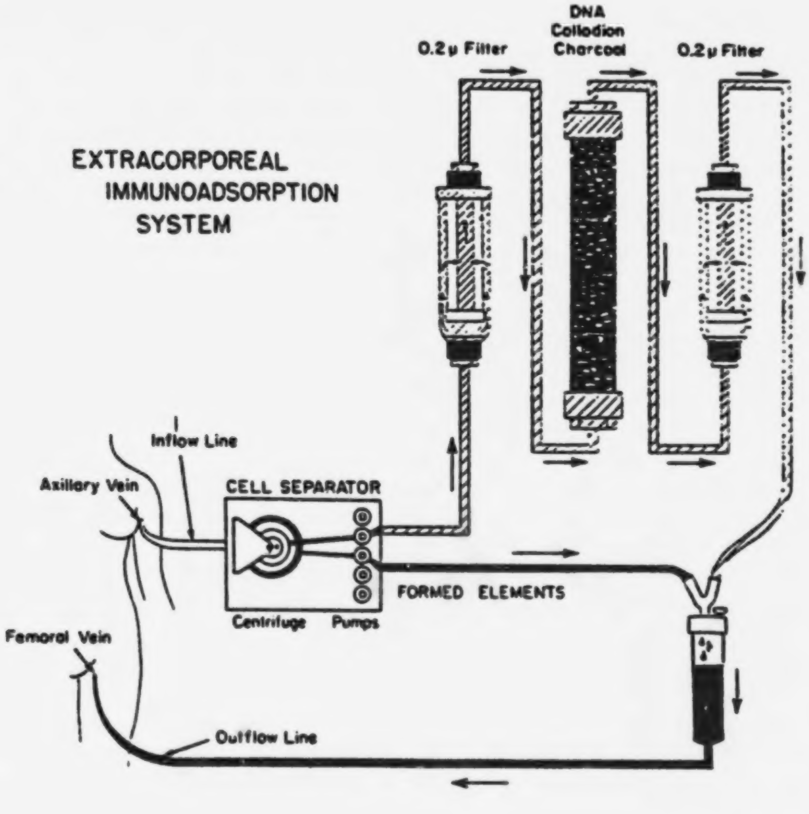
EXTRACORPOREAL IMMUNOADSORBENTS: USE IN HUMAN DISEASE

Systemic Lupus Erythematosus

In a previous section, the feasibility of extracting DNA antibodies by extracorporeal immunoadsorption over a DNA collodion charcoal (DCC) immunoadsorbent was demonstrated. The technology was used to selectively extract DNA antibodies from the serum of a patient with severe SLE. Concomitantly, extracorporeal filtration was employed to subtract immune complexes from sera (20). The extracorporeal system is schematically represented in figure 11.

A 29-year-old Caucasian woman with known lupus erythematosus was admitted for evaluation of nephrotic syndrome. In 1965 she presented with malaise, arthritis, dyspnea, and pleuritic pain. Over the next 5 years, she manifested malar rash, photosensitivity, alopecia, and pericarditis. In 1971, a renal biopsy showed focal proliferative glomerulonephritis. In 1975, the patient stopped her medications and was lost temporarily to followup. Physical examination on admission showed periorbital and pretibial edema and a faint pericardial friction rub. White blood cell and platelet counts were $4{,}000/mm^3$ and

Figure 11.--An extracorporeal circulation system employed for treatment of severe lupus nephritis


DNA
Collodion
Charcoal
0.2 μ Filter
0.2 μ Filter
EXTRACORPOREAL
IMMUNOADSORPTION
SYSTEM
Inflow Line
Axillary Vein
CELL SEPARATOR
FORMED ELEMENTS
Centrifuge
Pumps
Femoral Vein
Outflow Line

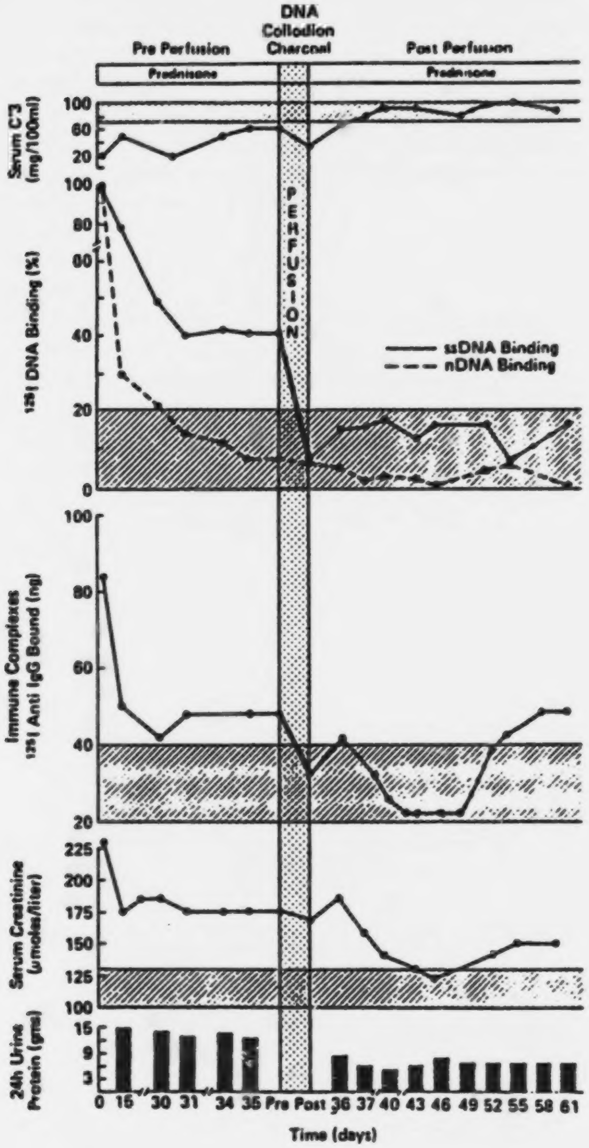
42,000/mm^3, respectively; BUN, 40 mg/100 ml; total protein, 4.9 g/100 ml; albumin, 2.1 g/100 ml; sodium, 40 mg/130 meq/ liter; potassium, 5.6 meq/liter; chloride, 117 meq/liter; and $CO_2$16 meq/liter. Chest X-ray showed a right pleural effusion and cardiomegaly. Urinalysis showed numerous red and white cells and pigmented granular casts. Antinuclear antibodies were positive at 1:64 titer with a homogeneous pattern. RNA-protein antibodies were negative. A renal biopsy performed on the 14th hospital day showed severe membranoproliferative glomerulonephritis with extensive subendothelial deposits.

On admission, the patient's serum showed abnormal levels of immune complexes, ssDNA, nDNA binding, and C3; creatinine and 24-hour urinary protein excretion were elevated (figure 12). By day 27, nDNA binding had declined to normal range; ssDNA binding, immune complexes, and creatinine had also declined, but reached a plateau at elevated levels by day 31 with no further change through day 35. Urinary protein excretion persisted between 12 and 15 g/24 hours for the duration of the preperfusion period. Prednisone was begun at 80 mg/day on day 1 and continued at this level throughout the entire study.

During the extracorporeal perfusion, 4,000 ml of plasma were processed through filters and DNA collodion charcoal for 3 hours. The amount of ssDNA binding in serum declined from 42 percent to 6.5 percent, and immune complexes declined from 48 ng to 32 ng of anti-IgG bound during the extracorporeal procedure. There was no significant change in nDNA binding. Eluates obtained from DNA columns at the conclusion of the experiments showed ssDNA binding of 46 to 86 percent, which was markedly reduced by preincubation with unlabeled ssDNA; nDNA binding in the same eluates ranged from 1.7 to 3.2 percent. A slight decline in serum creatinine and C3 occurred during the procedure. There was no significant changes in hematocrit, white blood cell count, total protein, sodium, potassium, chloride, magnesium, or uric acid. Hypocalcemia developed after 2 hours and 50 minutes of treatment due to the use of acid citrate dextrose as an anticoagulant and the binding of calcium to charcoal. That was corrected by calcium gluconate infusions. There were no untoward clinical effects from the procedure.

Binding of ssDNA and nDNA remained in the normal range for the 26 days after perfusion and did not rebound substantially. Immune complexes continued to decline over the 18 days after perfusion, associated with normalization of C3. During that period there was a progressive reduction in serum creatinine and a marked diminution in the 24-hour urinary protein excretion rate (figure 12). Fourteen days after perfusion, the patient developed an acute flare of arthritis, which was associated with an abrupt rise in immune complexes and a progressive rise in serum creatinine. The amount of ssDNA and nDNA binding remained unchanged during this exacerbation. Ibuprofin was added to her regimen, and that reduced the pain associated with her arthritis. On day 61, the patient returned from a pass with pneumonia and meningitis. Streptococcus group D was cultured from sputum, blood, and spinal fluid.

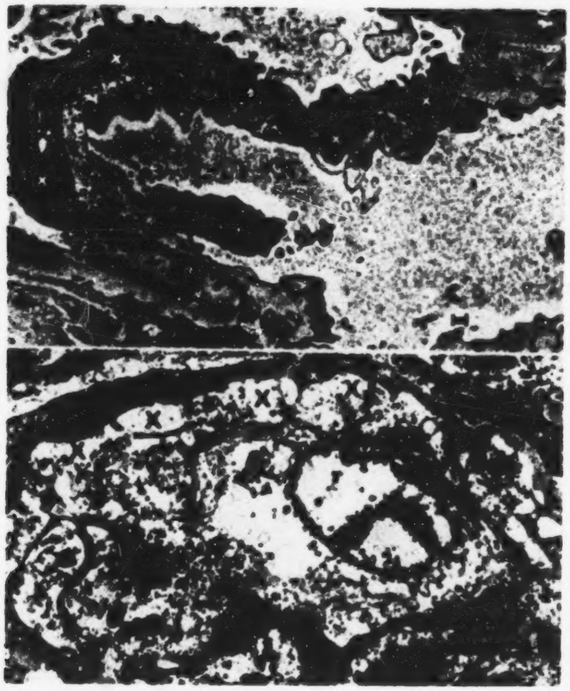
Figure 12.--Immunologic and renal functional parameters before, during, and after extracorporeal immunoadsorption over DNA collodion charcoal and filtration. Decline in ssDNA antibody and immune complexes was brought about by the extracorporeal procedure. In the postperfusion period, sustained reduction of ssDNA antibody and immune complexes was associated with progressive improvement in serum creatinine and proteinuria.



She did not respond to antibiotic treatment and died on day 62. Extensive postmortem examination of both kidneys showed a striking reduction in the amount of electron-dense subendothelial glomerular deposits from that observed on her initial renal biopsy (figure 13).

That was the first reported attempt to selectively extract anti-ssDNA antibodies and immune complexes from the circulation of a patient by means of extracorporeal immunoadsorption and filtration. The improvement in renal function in the days that followed the reduction in ssDNA binding and immune complexes in plasma by the extracorporeal procedure suggests that those substances were, in part, contributing to the glomerulonephritic process.

Figure 13.--Electronmicrographs of kidney biopsies obtained before and 28 days after extracorporeal perfusion, showing striking reduction of subendothelial glomerular deposits



Binding of ssDNA in serum was efficiently reduced by the extracorporeal adsorption, and a portion of that ssDNA binding was recoverable from eluates obtained from DNA collodion charcoal columns. The rebound in ssDNA binding in sera in the postperfusion period was minimal compared to that observed after specific antibody removal in actively immunized animals (27,24). In addition, the reduction in ssDNA binding was specific since nDNA binding was not changed significantly during the extracorporeal procedure. Indeed, the immobilization of calf thymus DNA in collodion charcoal, under the conditions employed, resulted in some denaturation of DNA, and, therefore, substantial quantities of ssDNA antigen were bound to the charcoal. Hence, the efficiency and specificity of ssDNA antibody removal, coupled with minimal rebound in the postperfusion period, suggest a role for this procedure in numerous other antibody-mediated diseases in which antigens may be available for immobilization on inert solid supports (5,25).

Immune complexes were measured by the solid phase Clq binding test, which has a high level of sensitivity. Elevated values have been shown to correlate with activity of disease in SLE (1). Immune complexes in serum were reduced to normal range by the extracorporeal procedure. Our preliminary studies showed that the 0.2-micron cellulose ester filter employed in the extracorporeal circuit could extract immune complexes from plasma of SLE patients that was passed through it, with minimal deleterious effects on other plasma proteins. Indeed, cellulose membranes have been previously shown to retain DNA:anti-DNA complexes (13) as well as various nucleotides and enzymes *in vitro* (14,17). Hence, it is likely that the observed reduction in immune complexes was effected by the 0.2-micron cellulose acetate filter in the extracorporeal circuit. In addition, the 0.2-micron filter served the function of trapping any platelets in plasma coming from the cell-separator, thus averting their potential aggregation and the release of vasoactive substances by the immune complexes that were forming on the DNA collodion charcoal.

A progressive decline in immune complexes was observed for 13 days after the extracorporeal perfusion; it corresponded to a reduction in serum creatinine and urinary protein excretion during the same time period. The decline in circulating immune complexes in the postperfusion period may be related to changes in the physical characteristics of residual complexes or perhaps to improved function of the reticuloendothelial system, which permitted their rapid elimination from the circulation. Indeed, recent studies have suggested that reticuloendothelial system immunospecific Fc receptor function is abnormal in SLE, and this defect may explain the elevated and prolonged circulation of immune complexes which may contribute to tissue deposition and damage (11,16). Reduction in ssDNA binding brought about by the extracorporeal immunoadsorption may have contributed to the continuing decline in immune complexes in the postperfusion period by providing less ssDNA antibody to combine with any free circulating ssDNA.

The resolution of electron-dense subendothelial deposits in the renal biopsy on day 62 reflected the ultrastructural counterpart of the observed renal functional improvement. Prednisone may have assisted in the resolution of renal inflammation, and its effectiveness may have been enhanced by the removal of the offending immune reactants in the circulation.

The acute onset of pneumonitis and meningitis as long as 28 days after extracorporeal perfusion was most likely related to sustained prednisone therapy, which rendered the patient susceptible to infection, rather than to the procedure itself. Indeed, infection is a well-established cause of death in patients with SLE who have been treated with immunosuppressive drugs for protracted periods.

Extracorporeal immunoadsorption as carried forth in our patient may selectively extract potentially pathogenic immune reactants with a single treatment and without the administration of costly replacement fluids other than saline, which is used to prime the extracorporeal circuit. The simplicity of the extracorporeal procedure and the lack of clinical toxicity or undesirable effects on hematological and biochemical parameters suggest that it may ultimately be an acceptable adjunct to drug therapy in certain cases of SLE that have shown an unsatisfactory response to pharmacologic agents alone.

PROSPECTS FOR THE FUTURE

Extracorporeal immunoadsorbents were initially developed as a means of efficiently extracting selected immune reactants from the circulation without the necessity of using plasma replacement. The concept has recently been brought to clinical application in patients with SLE and adenocarcinoma of the colon, and the initial results have been promising. Much of the outcome of immunoadsorbent treatment was initially considered to depend on the rate of replacement of the substance that was removed. Measures employing various immunosuppressive drugs and combination chemotherapeutic agents to suppress rebound in man and experimental animals have been tried, and promising initial data have been obtained. From the reported experience with plasmapheresis, the antibody rebound may not be as significant a problem in some human diseases as it is in actively immunized dogs (15,21). Indeed, in the SLE patient treated by extracorporeal immunoadsorption there was minimal antibody rebound in the postperfusion period after ssDNA antibody extraction. The development of instruments capable of manipulating the rebound phenomenon if it should occur would be quite desirable. Perhaps the optimal approach to prevent production of pathogenic antibody formation after extracorporeal adsorption would be to reinstitute a state of immune tolerance to the antigen. Indeed, some new modalities for induction of tolerance in the host with ongoing autoimmune disease have been described (3).

Of interest is the observation that extracorporeal immunoadsorption may subtract various inhibitory reactants and lead to stimulation of potent tumoricidal effects, as seen in dogs and in a single human patient. Further exploration is needed to carefully define the nature of the substances that are subtracted and the sequence of events that are set in motion by the process.

Autoantibodies directed to tissue antigens are now considered to be involved in numerous disease processes. There is a need to immobilize various cellular antigens in order to utilize them to extract tissue-specific antibodies in the circulation. Likely candidates for this process would be thyroid-stimulating hormone in Graves' disease and acetylcholine receptors in myasthenia gravis. Preliminary work has shown that GBM and erythrocyte antigens may be immobilized in extracorporeal circulation systems, and they are capable of removing their respective antibodies from plasma (5,25). Indeed, use of immobilized antibodies specific for various drugs as a means of reducing toxic levels in the circulation in cases of drug overdose might also be a fruitful endeavor.

Additional study of the use of immobilized enzymes for hydrolysis of various harmful substances in the circulation would be desirable, as would development of methods for removing immune complexes from the plasma. Refinement of our understanding of the basis of disease and the development of methods to simplify measurement of the pathogenic substances in plasma would make it possible to plan and to predict the appropriate points at which to introduce extracorporeal immunoadsorption. Refinement of blood separation technology is needed to make the technique easier to accomplish. Indeed, several new steps have recently been taken (7).

Finally, extracorporeal immunoadsorption may yield considerable light on the pathogenesis of disease processes by selectively eliminating those substances considered to be pathogenic in a given disease. Indeed, factors governing the regulation of production of those substances may also be better understood. Hence, extracorporeal immunoadsorption may offer numerous possibilities for improving our biologic understanding of disease as well as providing the clinician with improved capability for treating disease.

ADDENDUM

Our group has now studied this approach in dogs with spontaneous adenocarcinoma of the breast, an excellent model for human breast cancer (23, 41). In these studies a unique and profound necrotizing tumoricidal response was observed after extracorporeal perfusion over *Staphylococcus aureus* Cowans I. In addition, the major effector system in initiating this tumoricidal response and its source was, in large measure, elucidated from these studies (23, 40, 41).

We found that only dogs that underwent extracorporeal perfusion over protein-A-bearing SAC, but not non-protein-A-bearing strain *Staphylococcus aureus* Woods 46, showed a necrotizing tumoricidal response (23). These findings suggested that protein A was the active immobilized substance producing the tumoricidal reaction. This was then confirmed by immobilizing purified protein A in a collodion charcoal immunoadsorbent column and profound necrotizing tumoricidal responses were observed in dogs with breast cancer after extracorporeal perfusion treatment (40). This finding appeared to exclude the possibility that a nondescript bacterial cell wall component on the SAC was producing the observed tumoricidal effect. Further studies employing radiolabeled protein A or SAC in the column during *in vivo* studies showed no significant release of radioactivity after perfusion treatment; hence, the observed tumoricidal response could not be ascribed to release of immobilized protein A or SAC into the host (23,41).

Our studies have strongly suggested that the active tumoricidal factor was present in the plasma of these tumor-bearing dogs and that this substance may be identified as a tumor-associated antibody (TAA). That the tumoricidal factor was present in the plasma of these dogs derived from the following evidence: a) tumoricidal reactions were extremely rapid in onset beginning within 30 minutes to 4 hours after the completion of extracorporeal perfusion (23); and b) *in vitro* perfusion of plasma from tumor-bearing hosts over SAC and SAW columns revealed striking increases in TAA as measured by radioimmunoassay after perfusion over SAC but not SAW (40).

Evidence from our studies suggesting that circulating complement-dependent TAA was the major mediator of the necrotizing tumoricidal response derived from the following: First, the observed tumoricidal reactions in dogs were specific for tumorous tissue and spared normal uninvolved mammary tissue; second, TAA measured by indirect immunofluorescence after extracorporeal treatment over SAC increased in plasma within 4 hours after perfusion, while no such increment was observed after extracorporeal perfusion over SAW in the same dogs (23); third, microscopic, ultrastructural, and immunohistochemical evaluation of tumor tissue within 4 hours after perfusion over SAC showed extensive necrosis of tumor cells and multiple lytic lesions on tumor cell membranes associated with membrane deposition of canine IgG and C3 (23); and fourth, serum C3 levels in dogs undergoing tumoricidal responses were reduced for up to 48 hours after perfusion (23).

Our further studies showed that mere removal of up to 50 percent of plasma volume by phlebotomy of tumor-bearing dogs resulted in no observed tumoricidal effect. However, additional studies showed contact of tumor-bearing serum *in vitro* with a SAC column resulted in the release of TAA from a circulating immune complex (40). Sera from dogs with spontaneous breast adenocarcinoma, which contained low levels of TAA but elevated levels of immune complexes, were passed over either SAC or SAW columns. TAA levels in effluent samples from SAC columns measured by radioimmunoassay increased up to 550 percent above preperfusion levels, while Clq binding in the same serum samples showed a profound decline through only the first 10 to 25 ml tested, which was followed by a rebound and overshoot to 25 to 156 percent above pretreatment levels in subsequent samples tested. No significant changes in TAA and immune complexes in the same plasma were observed in effluent samples after perfusion over a SAW column. PAGE electrophoretic analysis of eluates from SAC and SAW columns which were incubated with the same tumor-bearing serum showed a substantially greater presence of light and heavy immunoglobulin chains in the SAC eluates. These findings suggested that TAA is liberated in tumor-bearing plasma after contact with SAC. This may be due to the subtraction of certain major immunoglobulin G molecules within circulating immune complexes by SAC, which may then result in the depolymerization of immune complexes and release of TAA (40).

Finally, we have described a necrotizing tumoricidal response in dogs with spontaneous breast adenocarcinoma which occurs after extracorporeal perfusion over immobilized SAC. The studies have shown that protein A is the active moiety on the SAC cell surface involved in the tumoricidal response. Further studies have suggested that the tumoricidal response is initiated by complement-dependent TAA which are present in plasma and activated by dissociation from circulating immune complexes after contact of the latter with immobilized protein A. The rapidity, magnitude, and specificity of the observed tumoricidal response suggests that this may represent a potent new immunotherapeutic approach to breast adenocarcinoma.

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DISCUSSION

Dr. Taswell: I was asked by Dr. Jones if he could present a few slides on this same subject, and if he would like to do that in 3 minutes-- Why don't you go ahead and just limit it to a very brief presentation, and then if Dr. Terman would come up to the table here, we will have the discussion period.

Dr. Jones: Well, this work is not exactly on this presentation, obviously, but we have been working in Seattle with some leukemic cats. We chose to use a different type of tumor, after reading Dr. Bansal's very eloquent paper on cancer.... Our cats have feline leukemia virus... spontaneously induced leukemia....

If I can have the first slide, I will show you that these cats are first subjected to a very low dose of irradiation. We have found in the mouse that low-dose irradiation removes a suppressor kind of activity. And then [after giving anesthesia] we remove 20 to 25 percent of their total blood volume. This is a kind of batch method, since we can't hook these cats up...to a cell separator.

[Slides]

So we have chosen, then, to centrifuge the blood and collect the cells and the plasma. The plasma then is run over the increasingly popular [staphylococcus column] and...remixed with the cells, and then readministered into the tumor bearer. This is done twice a week.

[Slide]

With this slide, we are monitoring here the feline leukemia virus state, as well as the circulating lymphoblasts; and these are the percent blasts of the circulating leukocytes.

And after irradiation, and then 1 week later, we initiate that immunotherapy that I have just described; and we see a drastic reduction in the number of circulating lymphoblasts. And after about 4 to 5 weeks, these cats revert from a virus-positive state to a virus-negative state.

I have this particular cat alive, now, at about 6 months. He remains virus-free, and also tumor-free. This level of activated cells is within normal [limits]....

[Slide]

This cat has experienced the same kind of response as did the previous cat, in the reduction; we see that the irradiation alone does not reduce the numbers of circulating lymphoblasts. We waited, here, for a longer period of time, but as soon as we initiated the immunotherapy, the extracorporeal removal of immunoglobulin, we see a drastic reduction in the numbers of circulating lymphoblasts. These remain low; and about 4 to 5 weeks later, he turns from virus-positive to virus-negative.

The way we monitor the virus is with an indirect immunofluorescence assay, and it is done blindly in another laboratory.

We have five cats--can I get the next slide, please--

[Slide]

--that we have treated thus far, and we see that the weight gain in these cats is remarkable. We see a cat here at 3.6 kilos at the beginning, and after several weeks, and removal of the virus and leukemia at this point in time, this cat has gained a tremendous amount of weight.

This one...is a very good responder, although he did not have drastic increase. We lost one cat to a massive hematoma, at this point in time, at 5 weeks. However, he had gone virus-negative at 4 weeks....

Okay. In the next slide, being trained as an anatomist, I must show you a few cells; and here we see what we are calling a leukemic cell.

[Slide]

That is the end of the slides. We think that this kind of result supports what Dr. Bansal has seen in the patient and in the dogs.... Thank you.

Dr. Taswell: Dr. Terman, would you like to get to a microphone?

Dr. Khan: These are obviously very fascinating and stimulating presentations, and both Dr. Terman and Dr. Bansal are to be congratulated.

My question is: Is it possible that some of the staphylococcal toxins may be released and get into circulation, for two reasons? One, some of the bacterial toxins have been shown to give tumor regressions in animals, in the past; and also, now we know, in light of some of the recent work with immune interferon, that [staph toxin] is one of... the good methods of producing immune interferon.

And indeed, if any of you have measured the interferon levels in these patients-- If not, that is one thing worth looking into, because the effects of immune interferon probably would be very similar to what you are seeing.

So I think this may provide a couple of alternatives that may explain such a rapid tumor destruction.

Dr. Bansal: Well, we did not look at the interferon, but your concern was in mind. We checked for the possibility of [staph toxins]. We obtained 9 liters of wash from the staph column, and concentrated it 10,000-fold to check for it. In addition to that, with a very sensitive test for techoic acid, that is, surface antigen on staphylococcus, was tested in all 19 dogs in the preliminary studies. And it was demonstrated that there was no techoic acid or antibodies to it, in dogs, and the studies were done by Professor Chagron (?) at George Washington University. So I was fairly convinced there was nothing coming off the staph, but there was no absolute proof that nothing ever came off the staph.

Dr. Terman: In support of that, we labeled the staphylococcus itself and embedded it in the filtration system, along with the unlabeled staphylococcus, then measured radioactivity on the column at 20-second intervals in the course of extracorporeal perfusion. And there was in essence no release of the material. The counts were stable throughout the 25- or 30-minute course, a rather reproducible finding. Further, no tumoricidal reactions were observed in dogs that underwent perfusion over *Staphylococcus aureus* Woods 46 (non-protein-A bearing) columns, suggesting that a nondescript cell membrane component on staphylococcus was inducing that reaction.

In addition, I think that would suggest, at least, that the protein and glycoprotein membrane constituents are not being shed into the host.

Dr. Pussell: Dr. Terman, you mentioned during the presentation of the dog work that there was a dramatic fall in complement levels following the perfusion. I wondered whether you attributed this to complement activation in the perfusion chamber, or whether this was consequent upon the fact the complement activation occurred within the chamber itself.

Dr. Terman: This is, I think, a very important point. There is a profound difference in serum C3 levels in the dogs perfused over *Staphylococcus aureus* Cowans I from those perfused over *Staphylococcus aureus* Woods 46 [as controls].... There is very little C^3 reduction in the control experiments, versus profound drops and sustained reduction in the study group.

The most obvious source of immediate complement activation in the study group would be on the SAC column itself, but the sustained reduction, I believe, would be attributable to consumption in a florid complement-fixing reaction in the tumor itself, as evidenced by the immunohistochemical studies in which the tumors were strewn with C3 within 24 hours after perfusion.

Dr. Pussell: I wonder what the effects are of perfusing animals with activated complement, under the circumstances, and whether it would be feasible to decomplement these animals prior to perfusion....

Dr. Terman: I believe complement activation is a key aspect of the reaction itself, an integral part of the mechanistic concept.

Dr. Pussell: I have one other question, and that is the effect of protein A on absorbing out immunoglobulins; it is specific in humans for classes I, II, and...IV....

What is the effect of IgG subclass IV in the patient, particularly [the patient] treated for the umbilical tumor? And whether the subclass IV may have specific antigen or effects that you are augmenting?

Dr. Bansal: Well, a lot of questions, and no answers. The first patient was just to show the feasibility. Very few studies were done concerned about anemia and the biochemical studies took priority and so forth, so a very limited number of immunological studies were done in this patient.

And at that time, not knowing some of the humoral changes, I had concentrated more on the lymphocyte population, which I thought...needed to be looked at. So therefore, these studies were not done, at least not by me....

Dr. Terman: No, we have not [done these studies]. We wish the canine reagents were available, but they are not. It certainly needs to be studied.

Dr. McLeod: We have had considerable experience with a "selective depletion" technique for the treatment of cryoglobulinemia. It consists of a series of plasma exchanges: In the first exchange, albumin or saline is used as the replacement fluid, but in subsequent plasma exchanges the patient receives autologous plasma, removed in the previous treatment, which has been depleted of cold-insoluble protein by incubation in the cold. We have treated 3 patients in this fashion and have observed a total of about 100 procedures, most of which have been done on an outpatient basis. This treatment process has been tolerated very well, and has been successful in reducing the patients' cryoglobulin levels and controlling symptoms of cryoglobulinemia.

I wish to ask the speakers if they see any particular disadvantage to selective depletion protocols of this sort, in which the plasma exchange step and the separation step are dissociated in time, and the separation step is performed on stored plasma? I believe this might have the advantages of using a somewhat less complicated apparatus for plasma exchange and of utilizing slower separatory processes, such as precipitation,which could not be applied in immediate on-line separation devices.

Dr. Bansal: Well, that depends on your belief. My belief was that it has to be done very rapidly, and that would only be achieved by a continuous separation. But Frank has already shown that a discontinuous method can be used; so perhaps both are possible.

Dr. Branda: As you know, plasma exchange in patients with malignancy hasn't given anything like the dramatic results you have seen, which suggests that the column is doing more than just simply manipulating immunoglobulin levels. I wondered if you have tried perfusing normal dog plasma through the column, and then putting that into an animal with a malignancy, to see whether it is just some change in plasma that is initiating this response.

Dr. Bansal: Yes. Tumor dogs are very difficult to get by; if you have one in 1-1/2 months, you're lucky. So I think once the principle has been established, then I think the second question, which you have raised, needs to be looked at. Of course, we went to a different process, and that is to use purified protein A, and the staph is not involved, to see if we get the same effect; then perhaps we can answer the question that you have raised, in a different mode.

At the same time, we would achieve a very simplified method. So I think it is a matter of approach. We are also concerned about the same question that you have asked, but approaching it differently.

Dr. Terman: And to answer it another way: If perhaps the controls in the dog work...showed no response, it doesn't answer your question as to the experiment that was not done; that is, with normal dog serum perfused....

Dr. Branda: Do those animals get the complement changes and so forth?

Dr. Terman: No, they don't. As I showed in the last slide, and I rushed through it, the complement drop is much less; it is slight, and then the rebound to normal levels is abrupt.

Dr. Branda: So it isn't quite the control.

Dr. Julian Breillatt: I thought Dr. Branda had asked my question, there, for a minute. It seems we're nibbling around the edges of what to me is the essential question. We started with plasma exchange. We know that Drs. Oon and Hobbs did a number of plasma exchanges for neuroblastoma, hypernephroma, and melanoma in England several years ago with minimal results until they added a cytotoxic agent to a specific antitumor antibody, and passively infused it after diluting the blocking factors.

Were one to do a simple plasma exchange, which should remove the blocking factors, it appears one doesn't get the results you do. Would you like to define the difference between plasma exchange and your specific depletion?

Dr. Bansal: Well, there are three very specific differences. Plasma exchange will remove those antibodies which may be beneficial. As you know, Isbister and Halliday had some beneficial effect by thoracic duct drainage, and Peter Alexander and Procter had the opposite.

The explanation of the beneficial effect was that serum blocking factors came out, and the explanation of the group in Chester-Beatty (?) was the beneficial antibodies were removed. So that is one objection.

The second objection is the time. The total procedure takes 60 minutes in dogs and 90 minutes in humans. It's a rapid exchange.

The third point is that staphylococci, the amount that we are using, could not remove any substantial amount of IgG. David has made that point again and again, that this couldn't be more than 200, 300, 400 mg at best.

The fourth point is that it is conceivable that there is a hyperregulation of IgG to the cell response. Initial studies of peripheral blood lymphocytes in dogs, using countercurrent distribution technique, suggest that there is a shift to the left; that means there is an increase in the B-cell population concomitant to a decrease in the suppressor cell population. However, the data are not quite confirmatory, and therefore I did not present any slide on that.

So there are some very distinct differences in the two approaches.

[The following presentation is an expanded version of comments given by Dr. Prasanta Ray during the discussion and later prepared in written form for this publication.]

Thank you very much for the opportunity to present some of our observations which have relevance to the papers presented by Dr. Terman and Dr. Bansal. We have been working for some time to develop and standardize a technique by which selective adsorption of immunoglogulin G and its complexes from the mammalian plasma becomes possible. Like Dr. Bansal and Dr. Terman, we also have been using heat-killed and formalin-stabilized Staphylococcus aureus Cowan I organisms for the immunoadsorption of immunoglobulin G and its complexes from the mammalian plasma. We have been able to circumvent most of the difficulties that Dr. Bansal mentioned in his description of treating a patient. We have developed a standardized technique and used it in three patients:

- An IgG-type multiple myeloma patient;
- A chronic lymphocytic leukemia patient having autoimmune hemolytic disorders; and
- A metastatic colon cancer patient.

The first patient we treated had an IgG-type multiple myeloma. Our attempt was to remove the abnormal IgG, and we found that immunoadsorption therapy could successfully remove larger concentrations of abnormal IgG molecules (table 1) from the patient's plasma, leading to a dramatic improvement of her general well-being. She was suffering from hyperviscosity syndrome and showed a significant decrease in the viscosity of the blood following immunoadsorption therapy. The patient had not responded to chemotherapy before the immunoadsorption therapy. Following the immunoadsorption therapy, the patient's condition improved; she gained back her strength, and could sit, walk, and take food by herself. She could not do that before the treatment. Chemotherapy was reinstituted at that phase, and the patient began to respond to it.

The second patient treated was a chronic lymphocytic leukemia patient having autoimmune hemolytic disorders. In that patient we used a combined technique of leukapheresis (for the removal of white blood cells) and immunoadsorption (for the removal of hemolytic antibody molecules). That treatment brought down his white blood cell (WBC) counts. His immunoglobulin G (hemolytic antibody) concentrations came down considerably (table 2). Two such treatments were given to the patient. His hematocrit gradually came up without any blood transfusion. His WBC counts were also lower following both treatments. At this stage, chemotherapy was reinstituted. The patient started responding to chemotherapy, which further lowered his WBC count to 54,000. After about 3 weeks his condition improved so much that he was released from the hospital. By 6 weeks, his hematocrit came up to 36 percent.

Thus, the lives of two terminally ill patients with IgG-related disorders had been prolonged by our technique. It may be relevant to mention that in the above-mentioned IgG-related disorders, the successful use of this procedure is being reported for the first time.

The third patient was a colon carcinoma patient with a metastatic lesion in the umbilical area. We subjected the patient to a series of vigorous immunoadsorption procedures. A total of 15 treatments were given altogether. During the initial four runs, her carcinoembryonic antigen (CEA) level came down from 1,700 ng/ml to 625 ng/ml. Then the CEA level increased a little, but eventually it came down again (table 3). Histopathological study showed fibrosis of the tumor with infiltration of a large number of lymphocytes.

In a DMBA-induced rat primary breast adenocarcinoma model (Ray, P.K., et al. Antitumor effect of *Staphylococcus aureus* organisms. *Fed. Proc*. 38(3): 1989, 1979), we have shown that producing immunoadsorption of plasma that has been separated from the anticoagulated blood drawn from the tail vein, with *Staphylococcus aureus* Cowan I, remixing it with the blood cells, and giving it back intravenously to the autologous tumor hosts, may lead to tumor regression and arrest of further growth of the tumor. Adsorption of the plasma by *S. aureus in vitro* and using it for an *in vitro* test for blocking immune complexes following the Hellstrom's technique, showed abrogation of the blocking activity. Further, the adsorbed plasma could show complement-mediated cytotoxicity against ^{51}Cr-labeled tumor target cells.

In a transmissible venereal tumor model, immunoadsorption of plasma immune complexes by *S. aureus* immunoadsorbent led to the regression of tumors. Some animals showed partial tumor regression, while others did not respond at all. Control animals, untreated tumor-bearing dogs, showed continued tumor growth. Mechanisms of growth inhibition in tumors in dogs are presently being studied.

In conclusion, our studies indicate that immunoadsorption of plasma with this particular strain of *S. aureus* could:

- Remove abnormal IgG's from a patient with IgG-type multiple myeloma;
- Remove autoimmune hemolytic antibodies from a CLL patient with autoimmune hemolytic anemia;
- Show partial tumor regression in a patient with a colon carcinoma; and
- Potentiate both cellular and humoral antitumor reactions against DMBA-induced primary rat mammary adenocarcinomas.
- Lead to the regression of progressively growing canine venereal tumors.

Table 1.--Ability of *S. aureus* suspension to adsorb out IgG from the plasma of the patient with IgG-type multiple myeloma *in vitro** and *ex vivo***

Plasma Sample	Concentration of IgG (mg/dl)***	
	In vitro adsorption	*Ex vivo* adsorption
Preadsorption	6,000	5,375
Postadsorption	3,600	2,875
IgG--bound to SA****	2,231	-

*For *in vitro* immunoadsorption, 900 ml of plasma of the multiple myeloma patient was passed *in vitro* through a capsulated bacterial filter (0.2 μ) containing 10% (w/v) *S. aureus* suspension. Samples collected before and after immunoadsorption were titrated for IgG.

**Patient's plasma separated in the celltrifuge machine was pumped through a capsulated bacterial filter (0.2μ) containing 10% (w/v) *S. aureus* suspension in a continuous manner. Samples collected before and after the procedure were analyzed for IgG.

***Measured by quantitative immunogel diffusion technique using Helena G-Quiplates.

*****S. aureus* cell suspension containing bound IgG was suspended in normal saline and sonicated (20 stroke/min) for a period of 3 minutes. The material was then centrifuged (3,000 rpm, 30 min) and the supernatant tested for IgG.

Table 2.--Gradual depletion of WBC's, platelets, and immunoglobulin G during the leukapheresis and immunoadsorption procedure*

					Immunoglobulin G	
Time	Hematocrit %	Lymphocytes	WBC Counts $\times 10^3$	Platelet Counts $\times 10^3$	Concentration (mg/dl)	Depletion %
Prerun	17	96	107.0	68	662	
30 min			96.0	47	188	71.6
60 min			84.3	41	312	52.9
90 min			76.1	35	475	28.2
Postrun	14	99	80.8	52	475	28.2

*The data represent various values after the second procedures.

Table 3.--Carcinoembryonic antigen (CEA) profile in a metastatic colon cancer patient undergoing immunoadsorption therapy using *S. aureus*

Number of Immunoadsorption Procedures	Concentrations of CEA in the Plasma of the Patient* (ng/ml)
None	1,700
One	1,500
Two	730
Three	665
Four	935
Five	820
Six	720

*The patient was given immunoadsorption treatment every 3 to 4 days. The general condition of the patient appeared normal. The patient has no complaints or complications, CEA level may indicate arrest of the disease.

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HYPERCHOLESTEROLEMIA TREATMENT BY HEPARIN-AGAROSE AFFINITY COLUMN

Bruce A. Kottke
Edwin A. Burgstaler
Howard F. Taswell
Ralph D. Ellefson
Larry D. Witte
Alvaro A. Pineda
Mayo Clinic and Foundation
Rochester, Minnesota

One of the most severe forms of atherosclerosis seen in clinical practice occurs in patients with the homozygous form of familial hypercholesterolemia. The disease is characterized by plasma cholesterol levels between 300 and 600 mg percent in heterozygous adults and by levels over 500 mg percent in homozygous children. The homozygous children develop xanthomas before age 10 and usually develop coronary and other vascular involvement prior to age 20 (13).

Recently, Goldstein and Brown have further characterized this disease. They have noted that whereas normal skin fibroblasts, smooth muscle cells, and lymphocytes have high-affinity-specific receptors for low-density lipoproteins following growth in lipoprotein-deficient serum, such receptors are absent in the cells of patients who are homozygous for familial hypercholesterolemia. Cells of heterozygotes have approximately 50 percent of the normal number of those receptors (7).

In patients with the disease, the serum cholesterol accumulation occurs almost entirely in the low-density lipoprotein (LDL) fraction (13). It has also been demonstrated that low-density lipoproteins accumulate in atherosclerotic arteries (8). Medical treatment of these individuals, especially those homozygous for the disease, has had limited success in normalizing their serum cholesterol levels (10). Because of that problem, other forms of treatment have been explored. One is ileal bypass surgery. The results of that procedure in homozygous patients have been variable, with lowering of cholesterol levels from 13 to 42 percent being reported (3,5).

Portacaval shunt surgery has also been reported to be beneficial in the treatment of several patients with homozygous familial hypercholesterolemia (4,14,15,21). While the procedure has been dramatically effective in some cases, concern regarding potential cerebral toxic effects has been expressed. During the past 2 years, it appears that enthusiasm for the procedure is waning.

Recently, two additional, novel approaches have been proposed and used in initial pilot studies: plasma exchange with replacement with a cholesterol-free plasma protein fraction using a continuous-flow blood cell separator; and removal of LDL and VLDL by affinity binding to heparin agarose. The first approach was pioneered by Dr. G. R. Thompson and associates at the Hammersmith Hospital in London (19,20). His results are detailed in his manuscript. Of particular importance has been his finding that angiographically demonstrated improvement of proximal coronary artery lesions occurred following 2 years of treatment of two homozygous patients with hypercholesterolemia (18).

The second approach was proposed by Doctor Lupien and associates (11). They have developed a system by which blood is drawn into transfusion bags containing heparin agarose beads in the presence of calcium ions, then returned to the patient via a high surface area filter system. They have shown that the heparin agarose beads specifically bind LDL and VLDL with little binding of HDL. They were able to achieve 15.6 and 19.3 percent reductions in serum cholesterol levels in two heterozygous patients with familial hypercholesterolemia. The results were achieved with no major side effects. They conducted preliminary studies in dogs and pigs which showed that except for the lowering of serum cholesterol levels and a modest rise in serum calcium from 10.0 to 10.8 mg percent and an increase in the prothrombin time from 18.4 to 28.8 seconds, no major side effects were noted. They monitored a large number of blood chemistries as well as white and red blood cell counts and platelet counts which were not altered by the procedure (2,12).

The specific removal of LDL and VLDL is a distinct advantage of the system since those lipoprotein fractions are thought to be the major ones that accumulate in atherosclerotic plaques (8). In contrast, HDL is thought to play a role in the removal of intracellular as well as extracellular cholesterol (1,16,17). The major drawback of the Lupien system is the relatively small volume of blood that can be treated in a reasonable period of time. My associates and I felt that disadvantage of the Lupien system could be overcome by combining the procedure with the use of the cell separator using an affinity column. That would allow the processing of large volumes of blood in a continuous flow system. In addition, it would markedly reduce the cost of plasma exchange since the patient's own treated serum could be utilized as the replacement solution rather than using the expensive purified human plasma protein fraction. We have estimated that this would reduce the cost of the procedure to 1/6 the cost of the usual plasma exchange. In addition, the potential for the development of immunologic incompatibilities in the patient would also be reduced when his own treated serum is used as the replacement solution.

Studies conducted by my associates have already demonstrated the technical feasibility of combining the use of affinity columns with the use of the cell separator (9). They have reported that using a column of charcoal-coated glass beads, they were able to achieve a 40 to 50 percent decrease of plasma bile acid levels and, after a second perfusion, a greater than 60 percent reduction of skin bile acid levels. The procedure relieved severe pruritus in all three patients studied.

To pursue this possibility, my associates have recently completed in vitro studies using plasma from a homozygous type II_a patient with familial hypercholesterolemia and also have conducted in vivo studies in dogs (6). For the in vitro studies, 500 ml of plasma was collected from a patient with homozygous familial hypercholesterolemia by plasmapheresis using heparin as the anticoagulant. The column used was 310 mm long with an internal diameter of 0.25 mm. The mean rate of removal of total plasma cholesterol was 22 percent per pass through the column. Ninety-five percent of the cholesterol removed was from the LDL and VLDL lipoprotein fractions.

Those studies also demonstrated that the column could be regenerated by eluting off the bound lipoproteins with 5 percent NaCl, and that regeneration could be repeated several times. During regeneration, the mean recovery of cholesterol in the eluate was 71 percent with a range from 44 to 90 percent. Chemical testing of serum sodium, potassium, phosphorus, total protein, glucose, alkaline phosphatase, SGOT, total and direct bilirubin, uric acid, and creatinine showed no changes as a result of perfusion of plasma over the column. The same results were seen if a column which had previously been regenerated with 5 percent NaCl was used. In comparisons of serum before and after perfusion through the column, manganese levels dropped a mean of 0.05 mg percent and magnesium 0.21 mg percent. The serum calcium level increased a mean of 42 mg percent. No cyanide accumulation was detected.

Encouraged by those studies, my associates then proceeded with in vivo studies in normal dogs. Two columns were arranged in such a fashion that one could be regenerated while the other was perfused with plasma obtained by plasmapheresis using the cell separator. A microfilter was incorporated into the return line to remove any particles or small fibrin clots from the column effluent. In two studies, the average volume processed was 1,117 ml, which resulted in a mean reduction of plasma cholesterol levels of 27 percent. In contrast to the in vitro studies, plasma calcium levels did not increase significantly. Thus, the system can result in continuous flow over an affinity column with the potential capability of processing 4 to 5 liters of plasma in 3 hours. The rechargeable feature of the system further contributes to a reduction of procedure time and cost.

Our most recent step has been to proceed with total plasmapheresis with purified plasma protein replacement in our homozygous familial hypercholesterolemic patient. The technique utilized is essentially that reported by Thompson and associates (19). In the initial plasmapheresis, the reduction in total plasma cholesterol was from 864 mg/dl to 264 mg/dl following 17 passes done over a 4-hour period. Over 95 percent of the cholesterol removed came from the LDL plus VLDL fractions. On the second plasmapheresis with 12 passes over 3 hours, the reduction in total plasma cholesterol was from 532 mg/dl to 282 mg/dl and again, over 95 percent of the reduction occurred in the combined LDL/VLDL fractions. It is of interest that even up to 3 to 4 weeks after plasmapheresis, the patient's plasma cholesterol did not return to preperfusion levels and has remained below 600 mg percent.

Preliminary in vivo animal studies by our associate, Dr. Witte, suggest that the heparin agarose column may remove the platelet-derived growth factor from plasma as well as removing LDL and VLDL. The platelet-derived growth factor is thought to play a major role in the stimulation of intimal smooth muscle cell proliferation at sites of endothelial injury. Thus, it may, at least in part, be responsible for the proliferative and fibrotic features of the atherosclerotic plaque. If removal of platelet-derived growth factor by the heparin-agarose column is confirmed by additional studies in animals and in patients, it would clearly represent a therapeutic bonus for the method in the treatment of severe hypercholesterolemia.

Our goal is to proceed cautiously toward the use of the column system in combination with the cell separator in patients with severe hypercholesterolemia. By comparing coronary angiograms before treatment and after several years of treatment, we hope to gain insight into the potential for lesion regression with this combined form of therapy. If such results are promising, a formal clinical trial that might include the treatment of several heterozygote patients with inoperable coronary disease will need to be considered as a distinct possibility. Certainly, if an effective means can be found to regress coronary atherosclerotic lesions in seriously ill patients with familial hypercholesterolemia, such a technique could be useful for a significant number of patients. At the present time, we do not have demonstrated effective measures for primary prevention in that subset of patients with coronary heart disease.

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DISCUSSION

Dr. Taswell: Thank you, Bruce. Are there questions for Dr. Kottke?

Dr. Michael Downing: One of the things that I noted in your talk, Dr. Kottke, was that you used heparin as an anticoagulant; and my first question will be: This endogenous heparin that is in the plasma that is now going through your column-- What effect will that have in competing for heparin that is insolubilized on your agarose?

If you could use some other sort of anticoagulant, perhaps you could increase the efficiency of your column and reduce the number of passes from 20, as illustrated in your slide, to only 1.

Dr. Kottke: I don't know if the effect would be that great, but we haven't tried this particular situation, that I am aware of, and perhaps Dr. Pineda can help me, if there are any other questions on it. As far as I know, heparin has been used correctly as the anticoagulant in all of these perfusions; so this is, perhaps, something worth exploring. Thanks very much, Mike.

Dr. Downing: And the other really isn't a question, it's a statement. If you go through the literature and look at particularly the blood coagulation field, you find that heparin-agarose has been used to isolate everything.

And I made a list of them, from memory: antithrombin III; platelet Factor IV, platelet-derived growth factor, which you mention; Factor IX, Factor X, Factor VII. I was wondering if you planned in the future to look at the effect of your procedure on the removal of these particular very important clotting factors from your patients' plasma.

Dr. Kottke: Yes; I would hope we would be able to do that, and I think with Ken Mann (?) and his associates here, we will be able to do some of those assays. I would hope we could explore it. I think it's a good question.

Dr. Pineda: We have been unable to use citrate alone. If you use citrate, formula A or B, you get clotting in the column. So we have to use heparin; I wish we could do away with it.

Dr. Thompson: I think that this is an entirely logical approach. In answer to the previous question, I am sure that your heparin-agarose doesn't remove more of all the various other things from plasma than plasma exchange does. And of course, what you are attempting to do is not so much to not remove other things, but to leave HDL behind. And if you achieve that, then that is theoretically a great advantage over plasma exchange; although on that last slide, it looked to me as if the decrease in HDL that you found in your in vitro study was identical to the decrease in LDL, and that is obviously something that needs looking into.

Before you respond to that, the interaction between LDL and heparin, of course, is an ionic interaction between a polycation and polyanion, and I am sure that heparin is not the only substance which might be used for binding LDL. There must be lots of other anions that will do exactly the same job, perhaps in the absence of calcium.

I think the fact that PPF costs so much in this country is a great advantage, because it will stimulate exactly this logical line of development, which we so need.

Dr. Kottke: Thank you very much for your comments, Dr. Thompson. I think your point is well taken that certainly, as time goes along, there are other absorbents, perhaps antibodies to LDL or other things that will bind the LDL, that might be more efficient, and this certainly needs to be explored.

In regard to removal of HDL, I think it does depend a little bit on how one calculates it. The problem is very simple. These patients have huge quantities of LDL, and their HDL is, in fact, very low. So while you may have a percentage reduction of HDL in terms of mass, it's a very, very small amount. But I agree that this does raise some questions of how specific the heparin-agarose is, and perhaps there would be better ways of keeping the HDL level high.

The fact that it removed the platelet-derived growth factor was a bit of a surprise, although if we looked at the literature carefully, we would have found that actually that has been used to purify this material to the degree that it is purified at present.

Actually, so far, as far as I am aware, with one study [in which they] thought they had it purified, it did not pan out in subsequent studies. To the present time, nobody has purified material with this binder.

One of the spin-offs of this is that it will be probable, for the first time, to measure the half-life of this factor by looking at its accumulation of mitogenic response after plasmapheresis. And we do plan to do this in our next studies.

Dr. Taswell: Are there any other comments or questions that anyone would like to make?

Dr. Taft: I would like to direct some comments toward some of the manufacturing representatives who are here. Many of the instruments on the market at the present time, and those in the foreseeable future, will not incorporate a number of desirable features.

First of all, the meters, which measure blood flow through the machines, are keyed to the pumps, and not to the actual blood flow. The blood flow that we calculate is at best a "guesstimate," and sometimes it can be in error by several hundred milliliters.

Secondly, we are increasingly using A-V shunts, or fistulas, or grafts, and therefore we have a direct method for blood pressure reading. It would be a great convenience for us to have, comparable to our dialysis colleagues, a direct blood pressure meter for those patients.

Finally, we have to weigh, by rather cumbersome means, the plasma that goes in and the plasma that comes out. A hand calculator is an essential piece of equipment to calculate for density, so one can calculate a reasonable estimate of volume. Therefore a tared balance for in and out would be a great feature to incorporate for plasma exchange.

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